

# Analytical Profiles of Drug Substances Volume 3

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## PREFACE

Although the official compendia list tests and limits for drug substances related to identity, purity, and strength, they normally do not provide other physical or chemical data, nor do they list methods of synthesis or pathways of physical or biological degradation and metabolism. For drug substances important enough to be accorded monographs in the official compendia such supplemental information should also be made readily available. To this end the Pharmaceutical Analysis and Control Section, Academy of Pharmaceutical Sciences, has undertaken a cooperative venture to compile and publish Analytical Profiles of Drug Substances in a series of volumes of which this is the third.

Reviews and comments received so far have reinforced our belief that the series fills a need and they have strengthened our determination to continue. The enthusiasm and cooperative spirit of our contributors have made these profiles possible. All those who have found the profiles useful are earnestly requested to contribute a monograph of their own. The editors stand ready to receive such contributions.

Beginning with Volume 2 a cumulative index has been added, to facilitate the correction of errors and to encourage the addition of relevant new information.

The concept of analytical profiles is taking hold not only for compendial drugs but, increasingly, in the industrial research laboratories. Analytical profiles are being prepared and periodically updated to provide physico-chemical and analytical information on new drug substances during the consecutive stages of research and development. Hopefully then, in the not too distant future, the publication of an analytical profile will require a minimum of effort whenever a new drug substance is selected for compendial status.

Klaus Florey

# **ACETAMINOPHEN**

*John E. Fairbrother*

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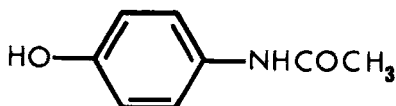
## ACETAMINOPHEN

### 1. Description

#### 1.1 Name, Formula, Molecular Weight

Generic names - Acetaminophen<sup>1</sup>,  
Paracetamol and Acetophenum<sup>2</sup>.

Chemical names - 4' - Hydroxyacetanilide; p-hydroxyacetanilide; p-acetamidophenol; p-acetaminophenol; p-acetylaminophenol; N-acetyl-p-aminophenol.



$C_8H_9NO_2$

Mol. wt. 151.16

#### 1.2 Appearance, Color, Odor, Taste

White, odorless, crystalline powder, possessing a bitter taste.

### 2. Physical Properties

#### 2.1 Spectra

##### 2.11 Infrared Spectrum

Infrared spectra of solid dispersions of acetaminophen in potassium bromide<sup>3,7</sup> and in Nujol<sup>6</sup>, have been recorded. In the solid state<sup>6</sup> the carbonyl stretching band appears at 1659  $cm^{-1}$  (1650  $cm^{-1}$ ; ref. 3), the N-H stretching band at 3326  $cm^{-1}$  and a broad O-H stretching band at 3162  $cm^{-1}$ . In solution the C=O, N-H and O-H stretching bands occur at higher frequencies.

<u>Solvent</u>	<u>C=O Stretching Band</u>	<u>N-H Stretching Band</u>	<u>O-H Stretching Band</u>
Chloroform	1686cm <sup>-1</sup> (9)		
Dichloro- methane	1690cm <sup>-1</sup> (6)	3435cm <sup>-1</sup> (6)	3588cm <sup>-1</sup> (6)
	1700cm <sup>-1</sup> (8)		
1,4-Dioxan	1692cm <sup>-1</sup> (8)		

Several other authors<sup>10,11,12,15,16</sup> report infrared spectra of acetaminophen. The infrared spectra of acetaminophen<sup>14</sup> in KBr and in a mineral oil mull are presented in figures 1 and 2<sup>13</sup>.

## 2.12 Ultraviolet Spectrum

The u.v. spectrum of acetaminophen has been recorded in a number of solvents, showing two bands in each. The long wavelength band corresponds to the  $A_{1g} \rightarrow B_{2u}$  transition while the short wavelength band corresponds to the  $\pi_N \rightarrow \pi_{CO}^*$  transition<sup>17</sup>.



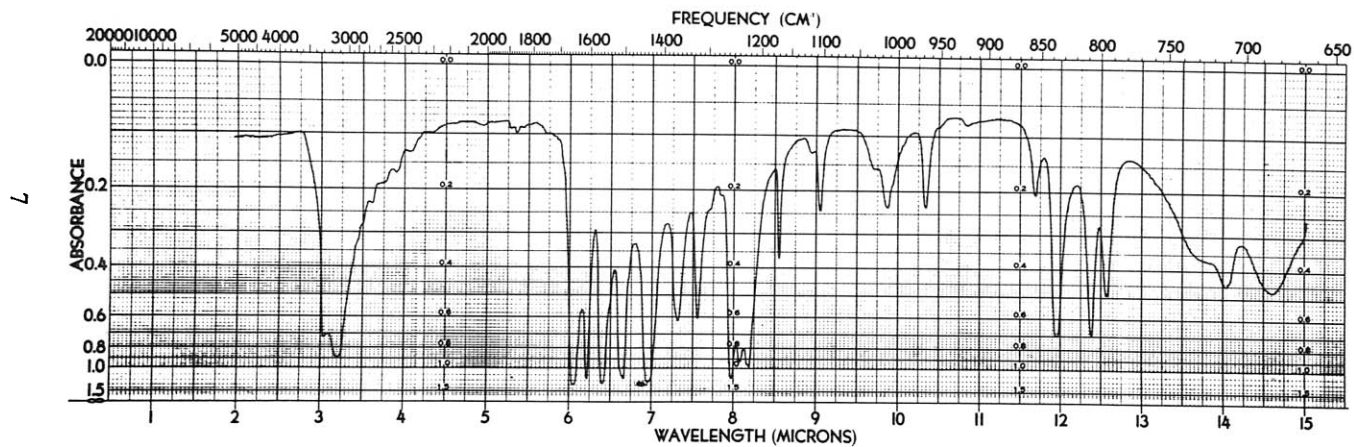


Fig. 1. Infrared spectrum of acetaminophen (KBr pellet)

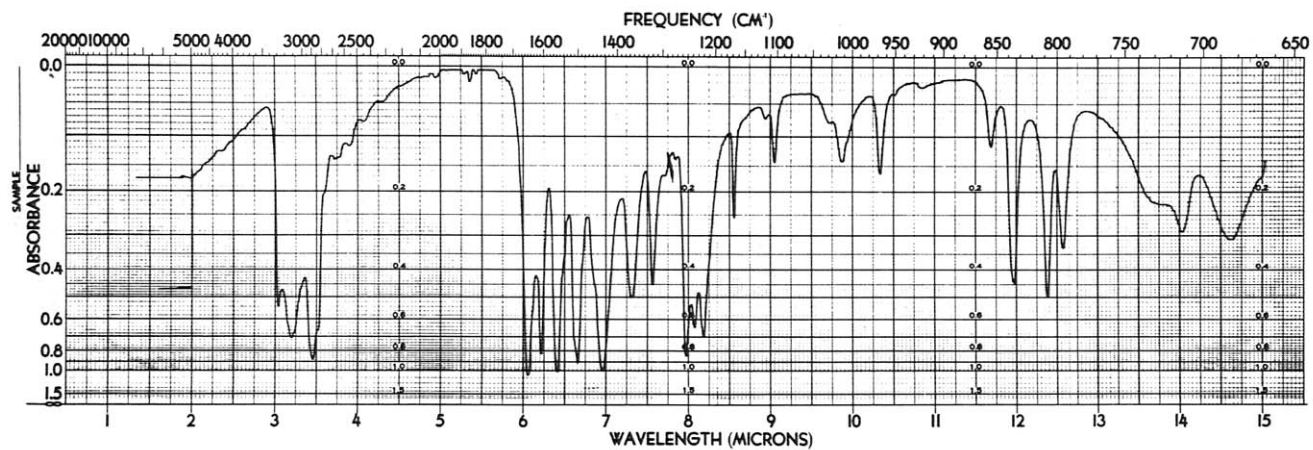


Fig. 2. Infrared spectrum of acetaminophem (Mineral Oil Mull)

## ACETAMINOPHEN

TABLE 1  
Absorption maxima of  
acetaminophen in neutral solvents

<u>Solvent</u>	<u>K band</u>	<u>B band</u>	<u>Reference</u>
Methanol	248-249m $\mu$ .		3,18
Ethanol (abs.)	249-250m $\mu$ .	about 290m $\mu$ .	4, 8,19
n-Butanol	250m $\mu$ .		20
iso-Propanol	250m $\mu$ .		19
Cyclo- hexane	244-245m $\mu$ .		19
Cyclo- hexane	278m $\mu$ .		8
Ether	264m $\mu$ .		19
Ether (dry)	247m $\mu$ .	about 283m $\mu$ .	8
Water	242.5- 243.5m $\mu$ .	about 283m $\mu$ .	8,19,23

The addition of acid to aqueous and alcoholic solutions does not give any observable change in the position of the maximum of the main band<sup>7,16,18,19,21,22</sup>. In 10<sup>-1</sup> M caustic alkali acetaminophen ionises to give the p-acetamidophenolate ion and the maximum of the main band is shifted bathochromically, in aqueous solution from 243 m $\mu$ . to about 258 m $\mu$ .<sup>19,20,22,23</sup> and in methanolic solution from 248 m $\mu$ . to 262 m $\mu$ .<sup>18</sup>.

TABLE 2  
Molar absorptivities ( $\epsilon$ ) of  
acetaminophen in different solvents

<u>Solvent</u>	<u>Wavelength</u>	<u><math>\epsilon</math>.</u>	<u>References</u>
Ethanol	249 m $\mu$ .	13,090 to 14,000	4,8,19,24
	288 m $\mu$ .	2,000 to 2,120	19,24
Methanol	249 m $\mu$ .	13,600	3
Ethanol/0.1N Hydrochloric Acid	249 m $\mu$ .	13,750	26
Water (pH 2 to 3)	242 m $\mu$ .	ca.11,000	25
Water (pH 7.2) (Clark and Lubs Buffer)	242.5m $\mu$ .	10,037	8
0.1N Sodium Hydroxide	257 m $\mu$ .	10,820	21
0.01N Sodium Hydroxide	258 m $\mu$ .	10,830	24
Water (pH 10 to 11)	258 m $\mu$ .	ca.10,500	25

The ultraviolet spectra of acetaminophen in ethanol (95%) and in 0.01N Sodium hydroxide (aqueous) are presented<sup>24</sup> in figures 3a and 3b.

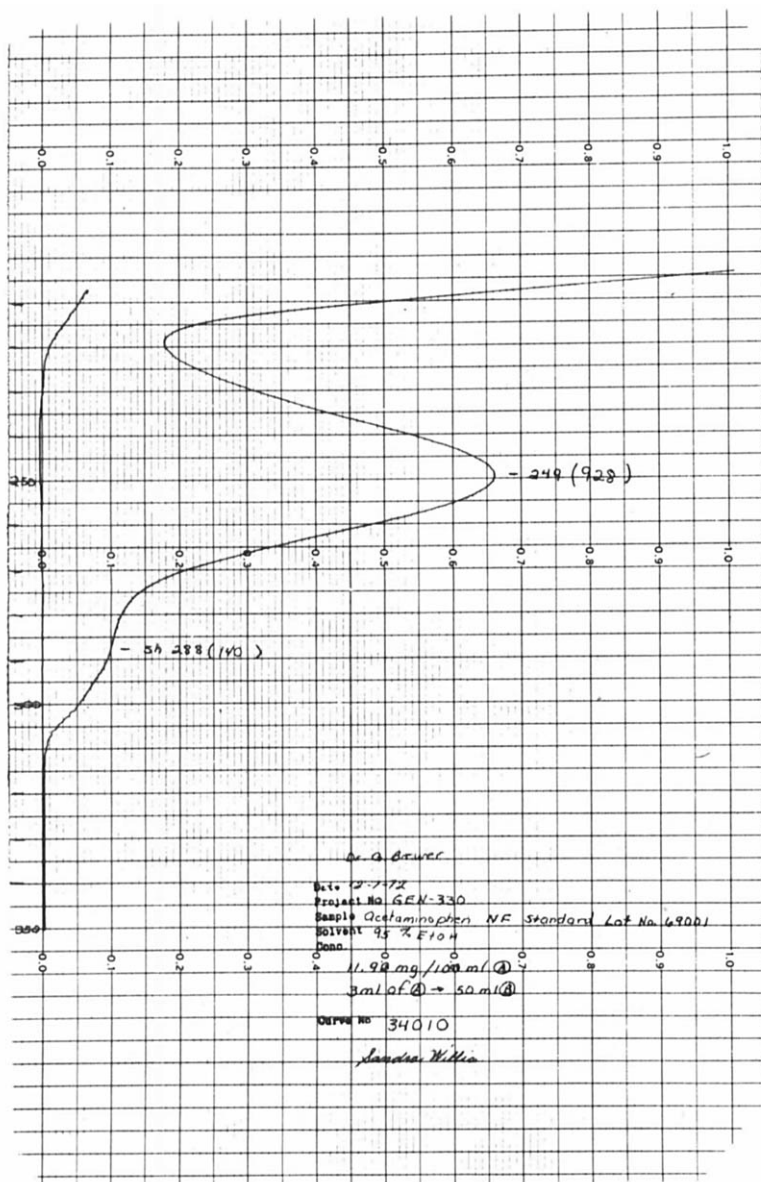


FIGURE 3a. Ultraviolet Spectrum of Acetaminophen (ethanol 95%)

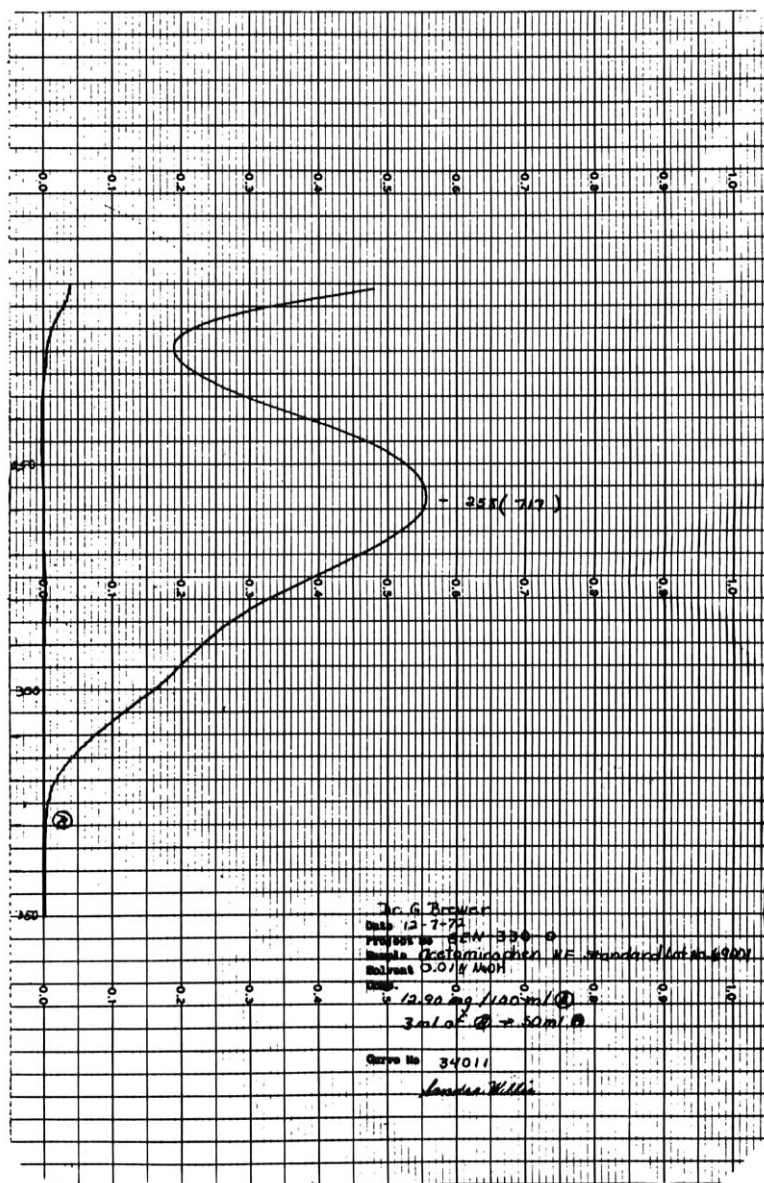


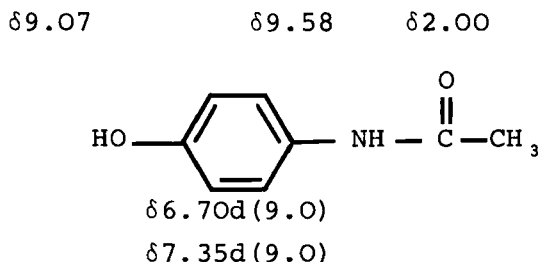
FIGURE 3b. Ultraviolet Spectrum of Acetaminophen (0.01N sodium hydroxide)

### 2.13 Fluorescence Spectrum

Acetaminophen has been reported<sup>25,27</sup> to exhibit fluorescence in neutral and acidic solution (excitation at 330 mμ. and emission peak at 400 mμ.). Nang et al.<sup>25</sup> also observed fluorescence in aqueous alkaline conditions (excitation at 315 mμ. and emission peak at 430 mμ.). However, recent attempts<sup>24,28</sup> to confirm these findings have been unsuccessful and it has been suggested<sup>28</sup> that the earlier observations<sup>25,27</sup> could have resulted from the Raman emission of water in conjunction with poorly aligned monochromator systems. Acetanilide is not fluorescent<sup>436</sup> and it is unlikely that the introduction of a para-hydroxyl group into the molecule would change this characteristic.

### 2.14 N.M.R. Spectrum

Puar and Funke<sup>201</sup> recorded the N.M.R. spectrum of acetaminophen in dimethylsulphoxide - d<sub>6</sub> (see figure 4) and assigned the observed chemical shifts in the following manner.



Theriault and Longfield<sup>181</sup> used the N.M.R. spectrum as determined in deuterated acetone to identify acetaminophen, formed by Amanita muscaria as a conversion product of acetanilide.

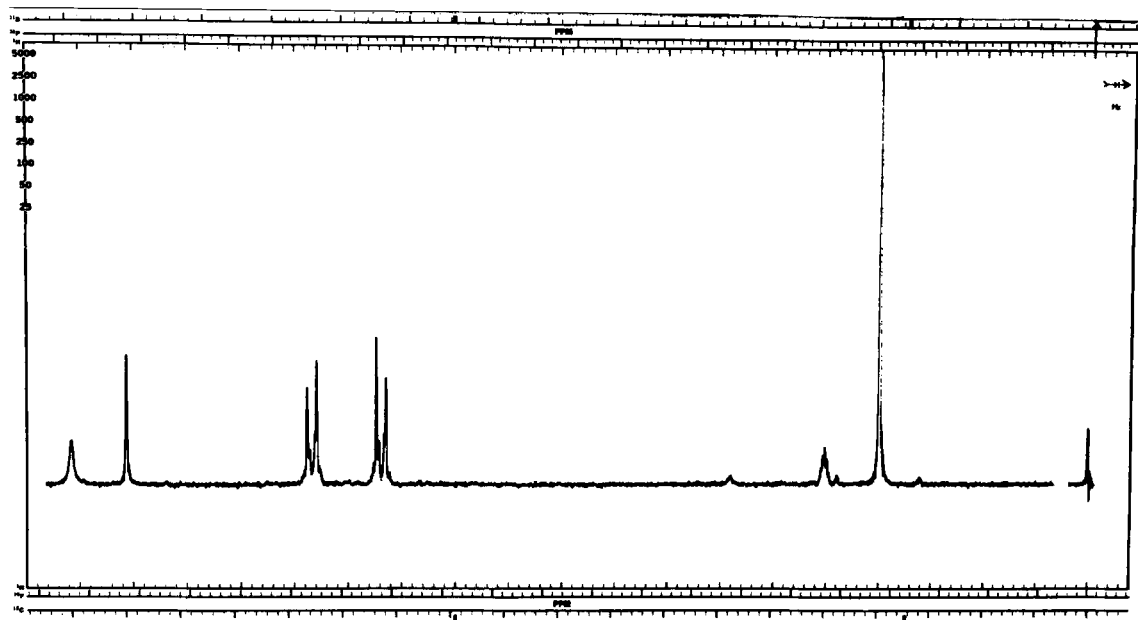


Fig. 4. N.M.R. spectrum of acetaminophen in deuterodimethylsulphoxide

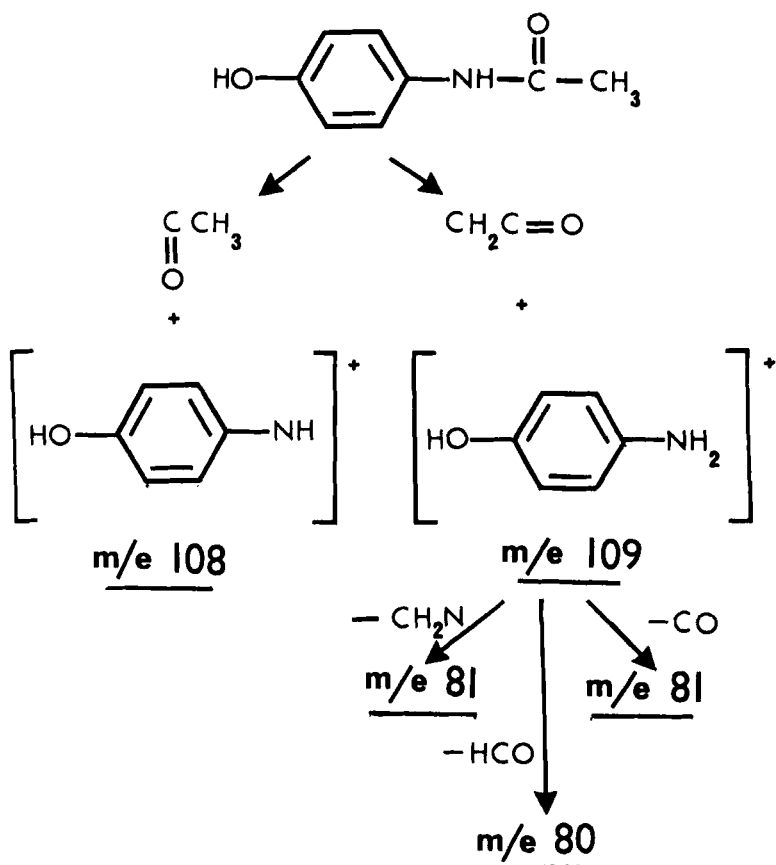


### 2.15 Mass Spectrum

The effects of substituents on the mass spectral fragmentation of para-substituted acetanilides has been studied in detail<sup>29,30,446</sup> but unfortunately examination of the p-hydroxy compound was omitted in each case. Burtis et al.<sup>11</sup> give the main peaks of the mass spectrum of acetaminophen as m/e 151, 135, 121, 109, 95, 81 and 55. The molecular ion undergoes a mass loss of 42 to give the base peak of m/e 109. This results from the re-arrangement of a proton of the acetyl group to the phenyl ring, followed by cleavage of the amide bond with the loss of  $\text{CH}_2\text{CO}$  (m/e 42). This metastable transition gives a strong diffuse peak.

Fales, Milne and Law<sup>444</sup> recorded the mass spectrum of acetaminophen, reporting the most abundant peaks as m/e 109, 151, 43, 80 and 81. The relative abundancies for m/e 40 to 152 are tabulated<sup>444</sup>.

Puar and Funke<sup>201</sup> have also recorded the high-resolution mass spectrum of acetaminophen (see figure 5) and suggest the following scheme of fragmentation:-



The occurrence of all three alternative modes of fragmentation of m/e 109 is supported by observation of corresponding metastable ions and high resolution data.

Milne, Fales and Axenrod<sup>445</sup> have recorded the isobutane chemical ionisation mass spectrum of acetaminophen indicating the peaks found to be m/e 152, 153 and 151.

# ACETAMINOPHEN

1595 ACETAMINOPHEN

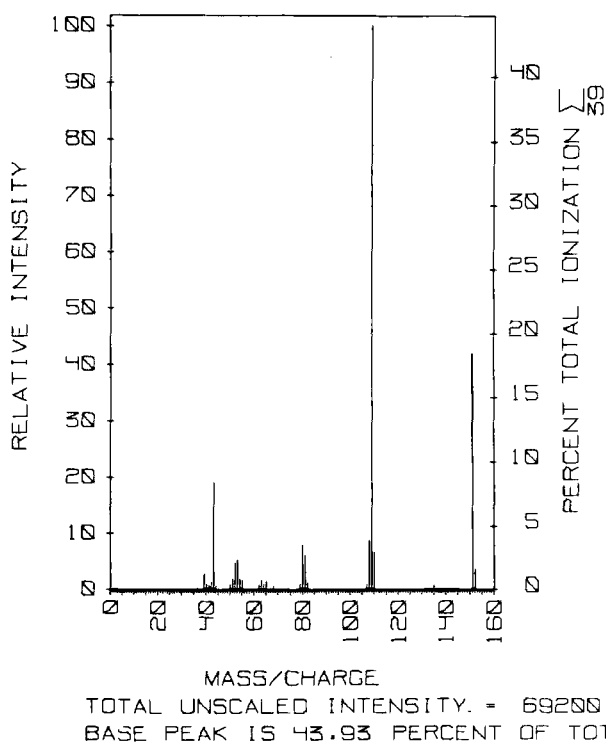


Fig. 5. Mass spectrum of acetaminophen

## 2.2 Physical Properties of the Solid

### 2.21 Melting Characteristics

The melting point first quoted<sup>31</sup> for acetaminophen (179°C) appears to be erroneous. Subsequent determinations gave melting points of 165 to 168°C for relatively unpurified material 32 to 39 and a melting range of 168 to 169°C for purified material 40 to 43. More recently improved purification procedures have been developed giving material which melts in the range 169 to 171°C.<sup>44</sup> to 48. It is this melting range therefore which is quoted in the current reference books<sup>3,4</sup> and official compendia 14,21.

Kuhnert-Brandstätter<sup>49,50</sup> has recorded the melting point using a Kofler hot stage as 167 to 169°C and carefully describes the melting process. From 140°C to the melting point grains, hexagonal prisms and rhomboids sublime. Residual crystals in this temperature range grow into hexagonal to polyhedral grains and prisms. The melt solidifies to a glass and gives unstable columnar aggregates at 110°C on which rectangular prisms of the stable modification are induced from about 140°C. This unstable modification (II) melts in the range 154 to 156°C.

### 2.22 Density

Fels<sup>40</sup> reported the specific gravity of acetaminophen at 21°C as 1.293.

### 2.23 Vapor Pressure and T.G.A.

Thermogravimetric Analysis (T.G.A.) failed to detect any loss of volatiles from a sample of acetaminophen N.F.<sup>430</sup>.

Jaeckel and Peperle<sup>432</sup> measured the dependence of the condensation coefficient

on the partial pressure over an evaporating crystal face of acetaminophen. Measurements of the vapor pressures on single crystal faces as functions of the partial pressure were made using a torsion balance.

## 2.24 D.T.A. and D.S.C.

Differential Thermal Analysis (D.T.A.) of a sample of acetaminophen N.F. gave<sup>430</sup> a sharp melting endotherm at 171°C. Examination<sup>431</sup> of a sample of B.P. grade material by Differential Scanning Calorimetry (D.S.C.) similarly gave an endotherm at 171°C. On cooling the sample and rescanning a different pattern was obtained showing the sample melting at 157°C and also an exotherm occurred at 67°C. From the D.S.C. data a value of 6.8 K cal./mol. was obtained<sup>431</sup> for the Latent Heat of Fusion.

## 2.25 Crystal Characteristics

Morse<sup>31</sup> in a paper describing the first reported synthesis of acetaminophen, recorded that it crystallised in the form of white monoclinic prisms.

Kuhnert-Brandstätter<sup>49,50</sup> has described visual changes which take place in crystalline acetaminophen during the melting process (see Section 2.21). Fels<sup>40</sup> obtained two apparently isomorphous crystalline forms of acetaminophen on recrystallisation from ethanol. From his optical examination of these crystals, Fels<sup>40</sup> assigns them to the monoclinic system with symmetry 2/m; C<sub>2h</sub>

$$a : b : c = 1.3688 : 1 : 1.5103$$

$$\beta = 115^{\circ} 49.5'$$

For the two isomorphous forms he<sup>40</sup> makes the following face assignments:-

	<u>Form 1</u>	m =	[110]
		c =	[001]
and	<u>Form 2</u>	r =	[101]
		m =	[110]
		b =	[010]
		a =	[100]
		c =	[001]
		f =	[130]

The observed angles between these faces are given and in some cases compared with calculated values. Form 1 is reported to be capable of undergoing transformation (to 001/010/100) but Form 2 does not.

This data has been systematised in the Barker Index of Crystals<sup>434</sup>.

Dispersion of the optical axes is very strong in acetaminophen,  $r < v$ . A very strong negative birefringence is exhibited.

Fairbrother<sup>449</sup> found that crystallisation of acetaminophen from a wide range of solvents gave essentially two types of crystal habit. Hexagonal prisms (by crystallisation from alcohols, esters, ketones, water, dioxan and acetonitrile) and slender rhombohedral needles (by crystallisation from benzene, toluene, dichloroethane and several other chlorinated solvents). Examination of these two crystal types by D.S.C., i.r. and x-ray diffraction (powder) failed to show any evidence of polymorphism.

## 2.26 X-ray Diffraction

Coy and Ochs<sup>433</sup> have recorded the x-ray powder diffraction pattern for a sample of acetaminophen N.F. (see Fig. 6 and Table 3).

# ACETAMINOPHEN

TABLE 3  
X-ray Powder Diffraction Pattern  
of Acetaminophen (7032-LKR-242)

<u>Interplanar Distances</u>	<u>Relative Intensities</u>
<u>d (Å)</u>	<u>I/I<sub>1</sub></u>
7.36	0.26
6.42	0.20
5.78	1.00
5.30	0.13
4.90	0.66
4.70	0.19
4.38	0.34
3.81	0.65
3.68	0.90
3.37	0.74
3.29	0.11
3.21	0.06
3.08	0.09
2.75	0.20
2.48	0.07
2.44	0.11
2.34	0.07

## 2.3 Powder Characteristics

### 2.31 Static Charge

Acetaminophen particles flowing through a hopper acquire a negative static charge<sup>51</sup>. This charge is reduced by the addition of tablet lubricants and by small quantities (0.5%) of water.

### 2.32 Flow Properties

The bulk density of acetaminophen granules falls with increasing water content. This represents a rise in internal cohesion and causes a deterioration in flow properties<sup>52,53</sup>. Spher-  
onization<sup>439</sup> of granules for tablet compression

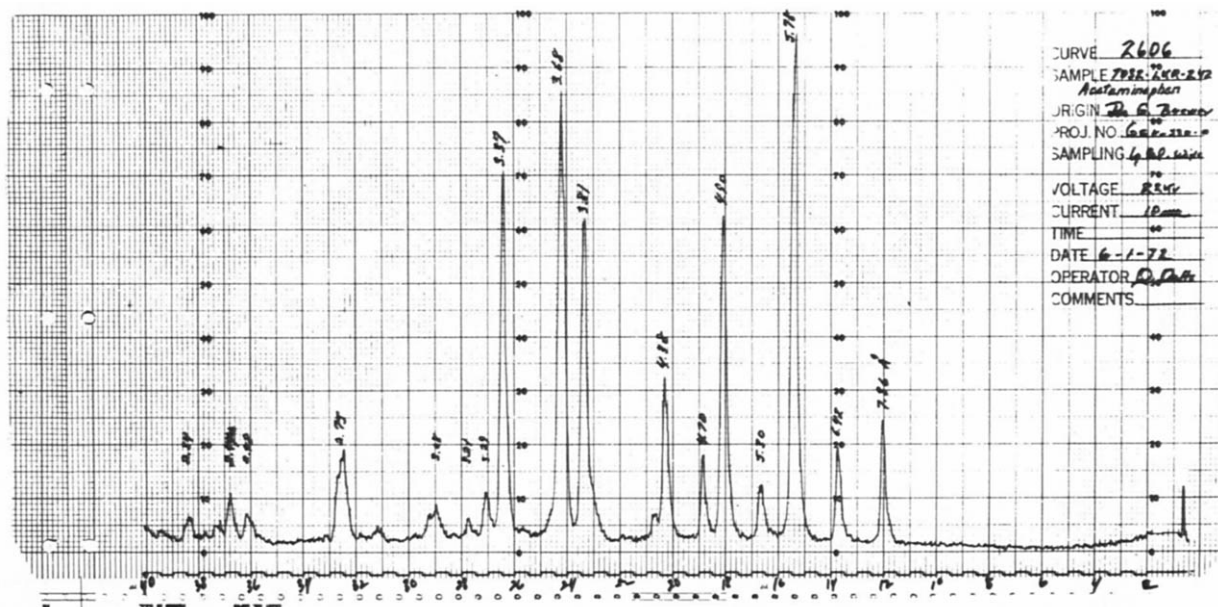


Fig. 6. X-ray powder-diffraction pattern of acetaminophen



improves granulation flow rate. The presence of water in acetaminophen granules increases the angle of repose<sup>52</sup>.

### 2.33 Compression Characteristics

Uniaxial compression of crystalline acetaminophen gives a pressure cycle typical of a Mohr's body<sup>55,59</sup> producing capping and laminating compacts. The effects moisture content and granulation have on the compression characteristics have been studied <sup>54,55,58,59,60,456</sup>.

### 2.34 Surface Area and Porosity

The surface area of acetaminophen powder compacts has been studied by the Brunauer-Emmett-Teller (B.E.T.) low-temperature nitrogen adsorption procedure<sup>56</sup>. The change of this surface area with changes in moisture content and/or compression pressure of the compacts has been studied<sup>57,58</sup>.

## 2.4 Solubility

### 2.41 Solubility in Aqueous Solvents

The solubility of acetaminophen in distilled water has been described by several authors.

<u>Temperature</u>	<u>Solubility (mg./ml.)</u>	<u>References</u>
20°C	about 11.3	61
	about 14.5	3,21,63
25°C	11.66	8,64
	13.85	65
37°C	about 19	66
	about 20	67
100°C	about 52	3,61,62,63

In pH 6.0 buffer solution at 37°C its solubility has been recorded<sup>68</sup> as 23.8 mg./ml.

Paruta and Irani<sup>69</sup> showed the solubility profile of acetaminophen in dioxan-water mixtures to correlate inversely with the polarity (dielectric constant) profile of the solvent for mixtures containing more than 30% water.

A similar study<sup>65</sup> conducted with sucrose solutions (as solvents) gave the opposite effect, the solubility of acetaminophen decreasing with decreasing dielectric constant of the solvent (i.e. with increasing sucrose concentration).

Goldberg et al.<sup>66</sup> examined the solubility of acetaminophen in aqueous urea solutions and found a linear increase in acetaminophen solubility with increasing urea concentration. This increased the solubility at 37°C from about 19 mg./ml. (in water) to about 31 mg./ml. (in 3.0 Molar urea solution). The authors<sup>66</sup> attribute the solubilizing effect to an interaction occurring in solution between urea and the acetaminophen. The solubility of acetaminophen in water is greatly increased in the presence of phenazone<sup>70,71</sup> by a process thought to involve hydrogen bonding. A similar situation is observed<sup>435</sup> in the presence of caffeine but theophylline has been shown<sup>435</sup> to reduce the solubility of acetaminophen.

#### 2.42 Solubility in Water Miscible Solvents

<u>Solvent</u>	<u>Solubility</u> (at 20°C)	<u>Reference</u>
Ethanol	1 in 10	63
Ethanol (95%)	1 in 7	21
Ethanol	1 in 8	61
Methanol	1 in 10	61,63
Acetone	1 in 13	21,63
Acetone	1 in 20	61
Propylene Glycol	1 in 9	21
		(cont'd)

# ACETAMINOPHEN

<u>Solvent</u>	<u>Solubility</u> (at 20°C)	<u>Reference</u>
Propylene Glycol	1 in 10	63
Propylene Glycol	1 in 50	61
Glycerol	1 in 40	21,63
Glycerol	1 in 50	61

The following solubilities have been determined under controlled conditions.

<u>Solvent</u>	<u>Temperature</u> (°C)	<u>Solubility</u> (mg./ml.)	<u>Ref.</u>
Water containing 2% ethanol	26.5	23.9	72
Propylene Glycol	37	156	68
Dioxan	25	90	69

## 2.43 Solubility in Solvents Immiscible with Water

<u>Solvent</u>	<u>Solubility</u> (at 20°C)	<u>Reference</u>
Chloroform	1 in 50	3,61,63
Benzene	Insol.	4,61,63
Ether	Insol.	3,61,63
Petroleum Ether	Insol.	4
Pentane	Insol.	4

The following solubilities have been determined under controlled conditions:-

<u>Solvent</u>	<u>Solubility at 37°C</u> (mg./ml.)	<u>Reference</u>
Cyclohexane	0.0015	67
Theobroma Oil	2.16	68

## 2.44 Rate of Dissolution

Dissolution rate studies conducted by Goldberg et al.<sup>66</sup> examined the dissolution of

monoparticulate layers<sup>73</sup> of acetaminophen, alone, and in fused and physical mixtures with urea. The dissolution of samples of pure acetaminophen followed pseudo-zero order kinetics over a 5 min. period. Coarse particles (50-60 mesh) gave data in reasonable agreement with the "cube root law"<sup>74</sup> thus representing a system requiring correction for the decrease in surface area during dissolution. Finer material (100-120 mesh) gave data more in agreement with a planar surface dissolution model. The eutectic and physical mixtures with urea gave biphasic dissolution curves, the rate constant of the first part being approximately twice that for pure acetaminophen of similar particle size, while the second rate constant closely resembled that for pure acetaminophen. This suggests the urea is leached out leaving a matrix of effective surface area comparable with that of the pure acetaminophen.

Mattok, McGilveray and Mainville<sup>75</sup> studied the dissolution of eight different lots of formulated acetaminophen tablets using the USP XVIII - NF XIII (rotating basket) method and two other methods<sup>76,77</sup>. None of the methods gave complete correlation with the blood and urine profiles obtained with the same samples. All of the recently manufactured samples required less than 15 mins. for 50% dissolution but stored samples showed greatly diminished dissolution rates in some cases.

Chow and Repta<sup>435</sup> studied the dissolution rate of acetaminophen and its physical mixtures and complexes with caffeine and theophylline. The anhydrous form and the monohydrate of the 1:1 acetaminophen-caffeine complex showed more than two and a half times the dissolution rate of acetaminophen. However, the hexahydrate of the acetaminophen-caffeine complex and the 1:1 acetaminophen-theophylline complex both showed a reduction in acetaminophen dissolution rate relative to pure acetaminophen.

## 2.5 Physical Properties of Solutions

### 2.51 Cryoscopy

Several eutectics of acetaminophen have been described in the literature:-

Eutectic with	Eutectic Temperature (°C)	Eutectic Composition (% Acetaminophen)	Reference
Phenacetin	115	-	49,50
Benzanilide	136	-	49,50
Urea	115	52	66
Acetylsalic- ylic Acid	118.2	37	85
Phenazone	83	28.5	6
	104	59.5	6

The cryoscopic properties of acetaminophen in naphthalene have been reported by Auwers<sup>86</sup>.

### 2.52 Ionisation and pH

Acetaminophen is a weak acid, its saturated aqueous solution having a pH<sup>14</sup> of 5.3 to 6.5 at 25°C. pKa values for acetaminophen have been quoted between 9.0<sup>78</sup> and 9.5<sup>80</sup> and also recently as 10.15<sup>435</sup>. Two papers describe the determination of the pKa value of acetaminophen by spectrophotometric procedures. Talukdar et al.<sup>19</sup> obtained a value of  $9.35 \pm 0.05$  (uncorrected for ionic strength) at 25°C, using the procedure described by Roth and Bunnett<sup>81</sup> and Dobáš et al.<sup>79</sup> a value of  $9.55 \pm 0.03$  (at 25°C) using the procedure described by Albert and Serjeant<sup>82</sup>.

### 2.53 Dipole Moment

The dipole moment of acetaminophen has been determined<sup>8</sup> in 1,4-dioxan solution using a Dipolmeter DMO1 (heterodyne beat apparatus) and

the molecular dipole moment calculated using the method described by Hedestrand<sup>83</sup>.

<u>Molecular Dipole</u>	3.99D
<u>Moment, <math>\mu</math></u>	

This result is in good agreement with the value of 3.96D reported by Lutskii et al.<sup>84</sup>.

Tomlinson's<sup>8</sup> value yields a Molar Orientation Polarisation ( $P_2^\infty$ ) of 325.4658 cm<sup>3</sup>.

Lutskii et al.<sup>84</sup> quote a value for  $\mu_{BZ}$  (i.e.  $\mu$  obs. -  $\mu$  calc.) of - 0.53 D .

## 2.54 Refractive Index

Microscopic studies with the Kofler hot stage<sup>49,50</sup> showed melts of acetaminophen to have a refractive index of 1.5403 at 174°C (for red light) and at 181 - 182°C (for sodium light). Using an Abbé refractometer solutions of acetaminophen in 1,4-dioxan and in methyl alcohol show linear increase of refractive index with concentration up to 3.6% w/w and 10.8% w/w respectively<sup>94</sup>.

From the equation:

$$n_{\text{(observed)}} = n_{\text{(acetaminophen)}}(x) + n_{\text{(methanol)}}(1-x)$$

(x = weight fraction of acetaminophen in solution)

a refractive index for acetaminophen of 1.608 (21°C, white light) was calculated<sup>94</sup> (methyl alcohol solution).

Measurement of the  $n_D$  in ethanol has been used<sup>87</sup> to quantitatively determine the concentration of acetaminophen in two component mixtures.

## 2.55 Adsorption from Solution

The quantitative adsorption of acetaminophen from 2% ethanol solution was investigated for the solid adsorbents, nylon, cellulose triacetate and cellulose by Ward and Upchurch<sup>72</sup>. The influence of temperature, time, solubility and solvent were examined.

Cellulose did not adsorb acetaminophen while nylon adsorbed almost twice as much as cellulose triacetate. Desorption studies indicated that adsorption occurred through hydrogen bond formation, the preferred mechanism being through the amido hydrogen of the acetaminophen and the carbonyl oxygen of the adsorbent. Brook and Munday<sup>89</sup> have examined the adsorption of acetaminophen on a dextran gel (methylated Sephadex (LH-20)) and suggest a similar mechanism of hydrogen bond formation.

## 2.56 Partition Coefficients

Acetaminophen is preferentially extracted into ether from acid and weakly alkaline aqueous solutions<sup>90,91,92</sup>. Brodie and Axelrod<sup>91,92</sup> examined the effect of pH on the partition of acetaminophen between ether and aqueous solution saturated with NaCl.

pH	Volume ratio (ether/water)	Fraction Extracted in ether phase	
		(ref. 92)	(ref. 91)
4.0	5	-	0.88
7.0	5	0.91	0.88
9.0	5	0.85	0.89
10.0	5	0.61	0.79
11.0	5	0.57	0.62
13.0	5	0.0	0.0

Partition coefficients for acetaminophen between other organic phases and water have been described.

Organic Phase	Partition Co-efficient (P)	Log P	Hansch Hydrophobic Substituent Constant ( $\pi$ )	Ref.
Cyclo-hexane	0.000075	n.a.	n.a.	67
Chloroform/ Ethanol	about 0.44	n.a.	n.a.	93
1-Octanol	-	0.55	-0.61	88
1-Octanol (partition with pH 7.2 buffer)	$6.237 \pm 2.0\%$	0.795	-0.36	8

Similar information may also be derived from the  $R_f$  values obtained in specially designed reversed phase silica gel thin layer chromatographic systems. Tomlinson<sup>8</sup> employed two systems of this kind. (see Section 6.27)

### 3. Molecular Complexes

Acetaminophen has been reported to interact with chloral<sup>95</sup> and with sorbitol<sup>96,97,98</sup>. Possibly these interactions may result in molecular complexes but insufficient data is available for any interpretation of this kind. A molecular complex of acetaminophen with pyrimidon (1:1) has been made<sup>99</sup> and acetaminophen is known to hydrogen bond onto the surfaces of nylon<sup>72</sup> and rayon<sup>72</sup>.



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Lach and Cohen<sup>100</sup> demonstrated the solubilisation of acetaminophen with alpha - and beta - cyclodextrins (Schardinger dextrins). The cyclodextrins exist in the form of cyclic chains having a relatively large open space within each molecule (6Å for alpha - and 8Å for beta - cyclodextrin). The interaction of acetaminophen with the cyclodextrins produces non stoichiometric inclusion complexes of the clathrate type<sup>101</sup>. Beta - cyclodextrin solubilises acetaminophen to a greater extent than alpha-cyclodextrin, the respective slopes of the interaction isotherms being 1.100 and 0.395.

When mixed with phenazone (antipyrine), acetaminophen was reported<sup>102</sup> to give a syrupy mass. Ridgway and Johnson<sup>70</sup> independently found that phenazone solubilised acetaminophen in water and that an equimolar molecular complex crystallised from solution. The complex was also obtained from alcoholic or acetone solution and from melts<sup>71</sup>. It showed a congruent melting point (109.5 to 110.5°C)<sup>71</sup> and a hydrogen bonded structure has been proposed by Dearden<sup>6</sup> for the complex.

Chow and Repta<sup>435</sup> prepared (1:1) complexes of acetaminophen with caffeine and with theophylline by a process of crystallisation from aqueous solution. These complexes were shown to exist in several hydrated forms:-

Acetaminophen Degree of (1:1) Complex Hydration with		M.pt. (°C)	K <sub>1:1</sub> (1.mole)	Heat of Solution at 25°C (K cal./ mole)
Caffeine	anhydrous	ca.145	59.4	6.5
Caffeine	monohydrate	75-80	-	10.1
Caffeine	hexahydrate	42-50	-	-
Theophylline	anhydrous	192-195	16.1	-

Theobromine does not form a complex with acetaminophen in aqueous solution<sup>435</sup>.

#### 4. Synthesis and Purification

##### 4.1 Chemical Synthesis

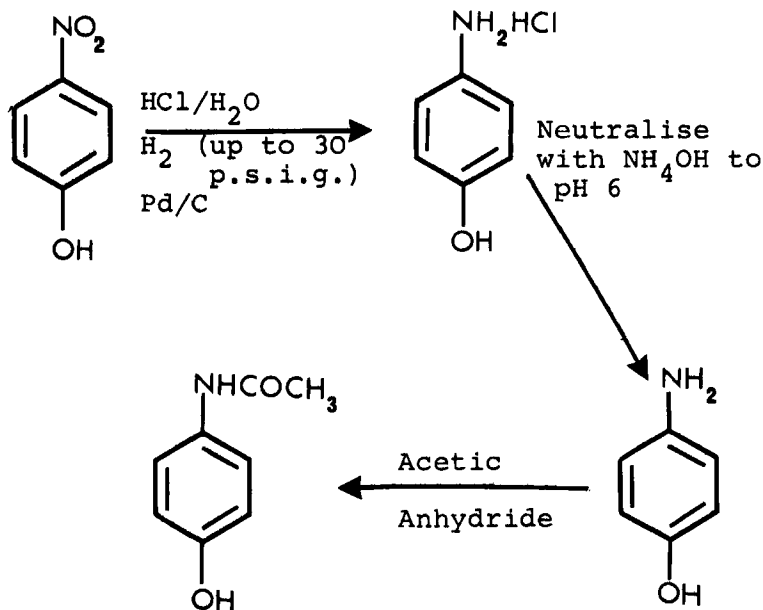
##### 4.11 Synthetic Routes

Acetaminophen was first synthesised by Morse<sup>31</sup> in 1878 by reduction of p-nitrophenol with tin in glacial acetic acid. The p-aminophenol produced by the reducing action of the tin was not isolated, being acetylated in situ by the acetic acid. Tingle and Williams<sup>35</sup> followed the Morse synthesis but found it necessary to increase the acetic acid concentration to 100% by the addition of acetic anhydride.

Vignolo<sup>103</sup> simplified the synthesis by employing p-aminophenol as his starting material which he acetylated with acetic acid. Friedlander<sup>32</sup> modified this process slightly by acetylating the p-aminophenol (from p-nitrophenol) with acetic anhydride in place of acetic acid. Many preparative methods have since been described employing the acetylation of p-aminophenol with acetic acid and/or acetic anhydride<sup>33,34,36,37,39,41,42,43,44,48,104,105,106,107,108</sup>; in some cases anhydrous sodium acetate also has been added<sup>34,36,37,41,42</sup>. The p-aminophenol has been produced in numerous ways including the electrolytic reduction of nitrobenzene<sup>44</sup>, the direct or catalytic hydrogenation of p-nitrophenol<sup>43,104,450</sup>, and the sulphide reduction of p-nitrosophenol<sup>107</sup>.

A typical<sup>43</sup> reaction sequence is:-

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In some processes the p-aminophenol is not isolated but is acetylated in situ as it is formed<sup>45,47,109,110</sup>. An alternative to the acetylation procedure uses the action of ketene<sup>111</sup> in p-aminophenol.

Other synthetic pathways involve the saponification of esters<sup>36,112,113</sup> such as 4-acetamidophenylacetate, the hydroxylation of anilides by chemical<sup>114</sup>, electrolytic<sup>115</sup> or enzymatic<sup>116</sup> processes, and the decomposition of diazo compounds such as p-AcNHC<sub>6</sub>H<sub>4</sub>N<sub>2</sub>BF<sub>4</sub><sup>117</sup>. Acetaminophen has also been synthesised from p-hydroxyacetophenone hydrazone<sup>118</sup>.

## 4.12 Purification

Crude acetaminophen is in most cases purified by recrystallisation from hot water<sup>39,45,48,110</sup>. Coloured impurities are removed during the recrystallisation by treatment with

activated charcoal<sup>39,48</sup> and oxidation is suppressed by the addition of small quantities of  $\text{NaHSO}_3$ <sup>110</sup>,  $\text{Na}_2\text{S}_2\text{O}_4$ <sup>45</sup> or hydrosulphite<sup>39</sup>. One process<sup>48</sup> controls the pH to 6.5 during the re-crystallisation by the addition of ammonia.

A number of processes seek to purify the p-aminophenol intermediate in a similar manner with activated carbon and  $\text{Na}_2\text{S}_2\text{O}_4$ <sup>43,44,437,438</sup> and in one case<sup>44</sup> also by extraction of the aqueous p-aminophenol with an organic solvent such as benzene, toluene, hexane etc., to remove impurities such as azoxybenzene and azobenzene.

A patent by Hahn and Quinn<sup>46</sup> deals specifically with the purification of acetaminophen and related compounds made from crude discoloured intermediates (p - aminophenol). The discoloured acetaminophen is dissolved in hot water, acidified (pH 1 to 5) with a non-oxidising mineral acid and kept in a non-oxidising atmosphere ( $\text{H}_2$ ,  $\text{CO}_2$  or  $\text{SO}_2$ ). The solution is agitated with activated charcoal, filtered and allowed to crystallise in the presence of an alkaline reducing sulphite, bisulphite, or hydrosulphite.

#### 4.13 Impurity Profile

The following impurities have been detected in acetaminophen:-

Substance	Origin	Amount in commercial (pharmaceutical) grade material	Reference
p-Nitrophenol	Synthetic precursor	-	119
p-Aminophenol	Synthetic intermediate	† 0.025%	7,14 (first suppl), 21
p-Chloro acetanilide	Impurity	† 10 p.p.m.	7,14 cont'd

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Substance	Origin	Amount in commercial (pharmaceutical) grade material	Reference
O-Acetyl para- cetamol (DAPAP)	Impurity from over- acetylation of para- cetamol	- none detected 1.1 to 1.3%	119 120 121
Azobenzene	By-products	-	44
Azoxybenzene	of reduction of nitro- benzene (precursor)	-	44
Quinone	Oxidation	Give a bluish	46
Quinonimine	of p-amin-	or greyish color	46
meri-Quinon- imine	ophenol (synthetic intermediate)	to acetaminophen	46
Inorganic Chloride		‡ 0.014%	7,14
Inorganic Sulphate	-	‡ 0.02%	7,14
Inorganic Sulphide	-	Not detected	7,14
Water	-	‡ 0.5%	7,14,21

## 4.14 Reference Standards

A National Formulary Reference Standard exists for acetaminophen<sup>14</sup>.

## 4.2 Biosynthesis

### 4.21 Metabolism of Phenacetin and Acetanilide

Acetaminophen is the main metabolite of both acetanilide and phenacetin (acetophenetidin) in man and in animals.

Acetanilide was introduced by Cahn and Hepp<sup>129</sup> as an analgesic and antipyretic in 1887. Investigating the metabolism of acetanilide, Mörrner (1889)<sup>122</sup> isolated potassium p-acetamidophenyl sulphate as a double salt with potassium ethyl oxalate from human urine. He also isolated a glucuronide tentatively identified as a conjugated p-acetamidophenol. Confirmation of this work was provided by Greenberg and Lester<sup>123,124</sup> and shortly after by Smith and Williams<sup>125</sup> who demonstrated that in the rabbit 70% of the administered dose was excreted in the urine as the glucuronide conjugate of acetaminophen and 12% as acetaminophen sulphate. The metabolic fate of acetanilide has since been studied in detail<sup>126,127</sup>.

In the case of phenacetin Mörrner<sup>128</sup> similarly isolated acetaminophen sulphate and the conjugated glucuronide from human urine. Smith and Williams<sup>130</sup> showed that 54% of the dose administered to rabbits was recovered in the urine as conjugated acetaminophen, (47% glucuronide and 7% sulphate). In man, Brodie and Axelrod<sup>91</sup> found up to 82% of the administered dose in the urine as conjugated acetaminophen and about 3% as free acetaminophen. More recent papers<sup>131,132</sup> give an essentially similar picture. A comparative study<sup>133</sup> of the availability of acetaminophen administered orally as such and as phenacetin gave availability ratios (acetaminophen/phenacetin) in two studies as 1.04 and 1.06.

#### 4.22 Prodrugs

Prodrugs are defined<sup>134</sup> as having physico-chemical properties different from the parent drug but retaining qualitatively identical pharmacologic effects and reverting to the parent drug in the body.

Acetaminophen forms numerous ester prodrugs<sup>67,135 to 154</sup>. Kotenko and Mokhort<sup>155</sup> describe an ethoxyphenylmethacrylamide homopolymer and its copolymer with o-carboxyphenylmethacrylamide which as analogs of phenacetin may be considered as prodrugs of acetaminophen. Extensive study has been made of the release of acetaminophen from 4-acetamidophenyl 2,2,2-trichloroethylcarbonate (ATC)<sup>67,134,136,148 to 153</sup>.

#### 4.23 Microbial Biosynthesis

Theriault and Longfield<sup>181</sup> studied the microbial conversion of acetanilide to acetaminophen. An unidentified Streptomyces species RJTS-539 gave a peak yield of 405mg./litre of acetaminophen from 1000mg./litre of acetanilide.

Amanita muscoria F-6 gave a mixed yield of acetaminophen and 2'-hydroxyacetanilide.

### 5. Stability

#### 5.1 Stability to Light

Acetaminophen is slightly light sensitive in solution<sup>63</sup> and may degrade by a mechanism involving pre-dissociation of the N-C bond as in the case of acetanilide<sup>171,172</sup>.

#### 5.2 Stability of Solid Acetaminophen to Heat

Dry, pure, acetaminophen is very stable at temperatures up to at least 45°C. Should it however, be contaminated with traces of p-aminophenol or be exposed to humid conditions such that hydrolysis to p-aminophenol takes place, then further oxidative degradation of the p-aminophenol occurs<sup>121</sup> characterised by a gradual color change through pink to brown and eventually to black. This involves the breakdown of the p-aminophenol to quinonimine and related compounds<sup>46</sup>.

### 5.3 Stability of Solutions of Acetaminophen

The degradation of acetaminophen in aqueous solution appears to be both an acid catalysed and a base catalysed reaction<sup>173,174</sup>. It is first order with respect to the concentration of acetaminophen and first order with respect to the hydrogen and hydroxyl ion concentration<sup>173</sup>.

Koshy and Lach<sup>173</sup> proposed reaction mechanisms for the acid and base catalysed hydrolysis of acetaminophen and determined the specific reaction constants ( $k'$ ) over the pH range 2 to 9.

pH	$k'$ (hours <sup>-1</sup> x 10 <sup>-4</sup> )			$E_a$ (K cal./mole)	$t_{1/2}$ at 25°C (years)
	35°C	70°C	90°C		
2	2.52	29.13	168.3	16.69	0.78
3	-	7.40	31.03	17.99	5.83
4	-	-	10.76	-	15.39
5	-	-	8.37	-	19.78
6	-	2.56	6.98	17.42	21.80
7	-	-	13.16	-	12.59
8	-	6.58	25.37	17.99	7.13
9	-	19.02	66.62	17.42	2.28

The above studies<sup>173</sup> were carried out isothermally. Zoglio et al.<sup>175</sup> repeated part of the study (pH 2 buffer) but used a nonisothermal linear temperature programmed technique. The results obtained were in good agreement with those of Koshy and Lach yielding a value for  $E_a$  of 17.0 K cal./mole and a value for  $k'$  (at 35°C) of  $1.95 \times 10^{-4}$  hr.<sup>-1</sup>. Zoglio et al. calculated the activation energy by comparing analytical data with Arrhenius model degradation curves using a digital computer. This approach was further improved by Kay and Simon<sup>176</sup> who recalculated the data of Zoglio et al. using an analog computer system.



#### 5.4 Stability to Oxidation

Acetaminophen is relatively stable to aerial oxidation unlike its hydrolysis product p-aminophenol. Acetaminophen has been used as an antioxidant for carotene in mineral oil solution<sup>177</sup>, a heat stabilizer for polyamides<sup>178</sup> and as an antioxidant, stabilizer and short-stopping agent for synthetic rubber latexes<sup>179</sup>.

#### 5.5 Compatibility with Excipient Materials

The compatibility of acetaminophen with a wide range of excipient materials has been reported<sup>156</sup> to <sup>170</sup>.

#### 5.6 Compatibility with Aspirin

Acetaminophen has been formulated in numerous commercial tablet preparations with aspirin. In some cases a third active drug substance such as caffeine, codeine phosphate or salicylamide is also present.

Acetaminophen is known<sup>85</sup> to form a eutectic product with aspirin (m.p. 118.2°C) and there is also some evidence to suggest that the two substances interact chemically to produce salicylic acid and diacetyl-p-aminophenol (p-acetoxyacetanilide).

Koshy et al.<sup>180</sup> found up to 4mg./tablet of diacetyl-p-aminophenol (DAPAP) in commercial products and studied the formation of DAPAP in laboratory prepared mixtures of acetaminophen and aspirin after storage for up to 1 month at 50°C. They also noted that magnesium stearate appeared to accelerate the formation of DAPAP.

Boggiano, Drew and Hancock<sup>120</sup> in a later study confirmed the formation of DAPAP in formulations containing acetaminophen and aspirin (compressed tablets and uncompressed mixtures) on storage at elevated temperatures (60°C). They

also suggested that codeine phosphate and magnesium stearate both accelerate the formation of DAPAP.

Kalatzis<sup>121</sup> refutes the findings of both the previous authors<sup>180,120</sup>, and shows DAPAP to be present as a synthetic impurity in commercial grades of acetaminophen and consequently is present in commercial products containing acetaminophen. In stability evaluation experiments with mixtures of acetaminophen and aspirin stored at 45°C for up to 2 months no DAPAP was formed. Samples of the acetaminophen/aspirin mixtures spiked with DAPAP in fact showed a gradual decline in DAPAP content if stored under humid conditions at elevated temperature.

### 5.7 Physical Incompatibilities

Acetaminophen shows physical incompatibility with antipyrine, Irgapyrin, Irgaphen, 2-phenylquinoline-4-carboxylic acid and diphenhydramine hydrochloride<sup>102</sup>, mixtures with these substances becoming sticky on mixing.

Rheological examination<sup>68</sup> of acetaminophen in microcrystalline cellulose-carboxymethylcellulose gels shows some evidence of an interaction between the acetaminophen and MCC - CMC.

Under humid conditions and at elevated temperatures acetaminophen discolours in the presence of codeine phosphate or caffeine<sup>121</sup>.

## 6. Analytical Chemistry

### 6.1 Identity Tests

Acetaminophen may be identified by its melting point<sup>50</sup> (see Section 2.21) and its eutectic temperatures with phenacetin<sup>50</sup>, benzanilide<sup>50</sup> or urea<sup>66</sup>. It may be identified by measurement of physical parameters such as infrared spectrum<sup>14,21</sup> or G.L.C. retention time<sup>182</sup>.

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Acetaminophen yields numerous derivatives many of which have clearly defined melting points:-

<u>Reagent</u>	<u>Derivative</u>	<u>M.p.</u> (°C)	<u>Ref.</u>
Benzoyl chloride - KOH	O-Benzoyl -acetaminophen	171	62,183
4-Nitrobenzoyl chloride-pyridine	O-(4-Nitroben- zoyl)-acetamin- ophen	210	21
Succinyl chloride -pyridine	bis (p-acetam- inophenyl) succinate	225- 227	135
Phthaloyl chloride -pyridine	O-Phthaloyl -acetaminophen	235- 237	137
Et <sub>2</sub> SO <sub>4</sub> - alkali	Phenacetin	134- 136	106
Allyl bromide	O-Allyl- acetaminophen	93	42
1-Fluoro-2,4- dinitrobenzene	p-(2,4-dinitro phenoxy)-acetan- ilide)	197- 198	184
Br <sub>2</sub> /CHCl <sub>3</sub>	2,6-dibromo- acetaminophen	174	41
conc.HNO <sub>3</sub> /conc. H <sub>2</sub> SO <sub>4</sub> (-5°C)	2-nitro- acetaminophen	158	185,186
Diazotised aniline - HCl	m-acetamino- o-hydroxyazo- benzene	226	34,36
1-Nitroso-2- naphthol-HNO <sub>3</sub>	10-acetamido- 5H-benzo- [a] phenoxa- zonium nitrate	337.5	190

Acetaminophen gives a characteristic violet-blue color reaction with a ferric chloride test solution<sup>3,14,21</sup> and may be distinguished from phenacetin by the color formed with Liebermann's reagent<sup>3,21</sup>. This involves oxidation of the acetaminophen with acid dichromate to slowly give a violet coloration in contrast to phenacetin which gives a red coloration.

Feigl<sup>187</sup> describes a spot test for acetaminophen claimed to have a sensitivity of 1 $\mu$ g. The test uses a procedure involving the nitrosylation of the amine group followed by its hydrolysis to a diazonium group which is subsequently coupled with 1-naphthol to give a red precipitate.

Le Perdriel et al.<sup>186</sup> found that in the case of acetaminophen the initial nitrosylation reaction proposed by Feigl did not occur but 2-nitro-4-acetaminophenol is being formed instead.

Acidification of the final Feigl test solution (containing 1-naphthol) (as applied to acetaminophen) produces a yellow-orange coloured solution whereas in the contrasting cases of acetanilide, phenacetin and p-aminophenol; red, violet and black precipitates are formed.

Paper and thin-layer chromatography have been used extensively to separate acetaminophen from other substances and the combination of R<sub>f</sub> value and chromogenic response to spray reagents may be used as an identity test. Of particular note are the papers by Gumprecht and Schwartzburg<sup>188</sup> (paper chromatography of isomeric monosubstituted phenols) and by Goenechea<sup>189</sup> (thin-layer chromatography of analgesics related to acetanilide).

## 6.2 Methods of Analysis

### 6.20 Gravimetric Procedures

Poethke and Köhne<sup>184</sup> describe the quantitative precipitation of acetaminophen with 1-fluoro-2,4-dinitrobenzene in a sodium bicarbonate-dimethylformamide medium to give p-(2,4-dinitrophenoxy) acetanilide. The precipitation is carried out over a 4 hour period and is claimed to give a precision of  $\pm$  0.3%. Caffeine, phenazone, 4-aminophenazone, phenacetin and codeine phosphate do not interfere.

## 6.21 Titrimetric Procedures

Acetaminophen may be determined by titration with sodium nitrite after prior acid hydrolysis of the acetaminophen to p-aminophenol. Both visual<sup>5,191,440</sup> and potentiometric<sup>192,193</sup> end-points have been used.  $\text{Ce}^{4+}$  quantitatively oxidises acetaminophen thus rendering it possible to titrate acetaminophen with  $0.1\text{N Ce}(\text{SO}_4)_2$  in an ethanolic HCl medium<sup>193,194</sup>.

Chatten and Orbeck<sup>195</sup> attempted to titrate acetaminophen with perchloric acid in various acetic anhydride based solvents but were unable to obtain an end-point.

Acetaminophen may be successfully titrated in a dimethylformamide medium with  $0.1\text{N}$  sodium methoxide (in benzene-methanol). The end-point may be determined visually using an azo violet indicator<sup>192</sup> or potentiometrically<sup>196</sup>.

Laurent<sup>197</sup> also using dimethylformamide solution titrated acetaminophen visually to a thymol blue end-point employing  $0.1\text{N Me}_4\text{NOH}$  (in benzene-methanol) as titrant. Fogg et al.<sup>20</sup> employed a similar system with  $0.1\text{M Bu}_4\text{NOH}$  as titrant, a  $\text{N}_2$  atmosphere and potentiometric end-point detection using a calomel reference electrode filled with  $\text{Et}_4\text{NBr}$  saturated dimethylformamide.

## 6.22 Polarographic Procedures

The anode polarographic behaviour of acetaminophen has been studied<sup>198</sup> at the wax-impregnated graphite electrode<sup>199</sup>. This system employs a solution of acetaminophen in aqueous ethanol/phosphate buffer (1:1), pH 7.1 and gives a value for  $E_{\frac{1}{2}}$  vs. S.C.E. of 333mV. Brockelt<sup>200</sup> describes a cathode polarographic procedure for the determination of acetaminophen after nitration with  $5\text{N HNO}_3$ . The solution containing nitrated acetaminophen is treated with potassium hydroxide and phosphoric acid to give a

solution pH of 5.8 and examined polarographically ( $E_{1/2}$  versus S.C.E. - 0.38V).

Shearer et al.<sup>441</sup> found that with the use of a glassy carbon electrode, acetaminophen could be determined polarographically with a peak potential of about + 0.5V versus S.C.E. This procedure is capable of selectively determining acetaminophen in the presence of p-aminophenol ( $E_{1/2}$  versus S.C.E. + 0.2V) and thus may be used as a stability-indicating assay. The water content of the acetate-acetic acid-methanol supporting electrolyte significantly alters the measured peak current for a given concentration of acetaminophen and thus has to be limited.

#### 6.23 U.V. Spectrophotometric Procedures

The British Pharmacopoeia 1963<sup>223</sup> and U.S. National Formulary XI<sup>62</sup> both adopted u.v. spectrophotometric procedures for the determination of acetaminophen in acetaminophen tablets. In both procedures the tablets are extracted with an anhydrous alcoholic solvent (B.P.-ethanol and N.F.-methanol), the extract acidified with a small amount of dilute hydrochloric acid and then further diluted with the alcohol. The acetaminophen concentration is determined by spectrophotometric measurement of the extinction of the solutions (249 m $\mu$ .) and its content calculated against, a standard E (1 percent 1 cm.) (B.P. method) or, the extinction given by a sample of the N.F. Reference Standard.

Brown and Gwilt<sup>26</sup> challenged the official B.P.<sup>223</sup> method on the grounds of cost of the solvent and the use of a standard extinction coefficient. They proposed the adoption of an alternative procedure employing 0.01N NaOH as both extractant and spectrophotometric solvent (extinction measured at 257 m $\mu$ ). This procedure was subsequently adopted for use in

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the Addendum 1964<sup>224</sup> to the B.P. 1963 and has been continued in the B.P. 1968<sup>21</sup>.

Rogers<sup>202</sup> examined the effects of slit-width on the precision of the B.P.<sup>224,21</sup> assay and calculated that for an extinction error of 0.2% a maximum half-intensity slit width of 1.7mμ. may be used (calculated for Hilger and Watts, Uvispek H.700 and Unicam SP. 700 spectrophotometers).

The U.S. National Formulary (XII<sup>203</sup> and XIII<sup>14</sup> editions) retains the use of the acidified methanol solvent but employs a revised extraction procedure in which the acetaminophen is extracted from an aqueous suspension of the ground tablet with a mixture of chloroform and ethanol (3:1). Ivashkiv<sup>93</sup> has studied the parameters of this<sup>14</sup> procedure as applied to the assay of Squibb Acetaminophen Tablets and reports the (75:25) ratio of chloroform to ethanol to be critical. Also examined were the partition coefficient of acetaminophen between the solvent phases (see Section 2.56) and the effect of the grinding procedure used, on the extraction of the acetaminophen. The results obtained indicate micro-milling should not be employed in the sample preparation.

Acetaminophen has been determined spectrophotometrically (250 mμ.) after partition into n-butanol<sup>20</sup> from sodium bicarbonate solution. This facilitates the determination of acetaminophen in the presence of aspirin<sup>20</sup>. In a similar manner acetaminophen may be determined in formulated tablets of the acetaminophen-phenazone complex<sup>204</sup> (see Section 3) by selective retention of the acetaminophen in 0.1N sodium hydroxide solution after partitioning with chloroform. The alkaline solution containing the acetaminophen is acidified with hydrochloric acid and the acetaminophen determined spectrophotometrically at 245 mμ.

Differential spectrophotometry has been used to determine acetaminophen in mixtures with other drug substances. Häberli and Béguin<sup>205</sup> determined acetaminophen in mixtures with salicylamide by differential spectrophotometric measurement at two wavelengths (255 and 301 mμ).

Shane and Kowblansky<sup>78</sup> used differential spectrophotometry to determine acetaminophen in the presence of aspirin, salicylamide and caffeine in analgesic tablets. Their procedure is based on the observation that the subtraction spectrum obtained by measuring the u.v. absorption of the p-acetamidophenolate ion (pH 10) against p-acetamidophenol (pH6) yields an absorption maximum near to the isobestic point of zero absorbance for salicylamide (263.5 mμ.). Under the same subtraction conditions caffeine and aspirin (which is converted to sodium salicylate) do not exhibit any absorbance from 255 to 340 mμ.

Routh et al.<sup>206</sup> employ a similar procedure for the determination of acetaminophen in the presence of aspirin and salicylic acid.

Acetaminophen has been determined spectrophotometrically in mixtures with other drug substances by several procedures involving preliminary ion-exchange (see Section 6.25) or partition chromatographic (see Section 6.26) separation of the acetaminophen.

#### 6.24 Photocolorimetric Procedures

The majority of the published colorimetric methods for the determination of acetaminophen are based on one of three systems. These are nitration, oxidation or hydrolysis to p-aminophenol followed by diazotisation and phenolic coupling.

Girard<sup>185</sup> nitrated acetaminophen with a mixture of nitric and sulphuric acids at -5°C



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to yield 2-nitro-4-acetamidophenol. Horn<sup>225</sup> described the nitration of phenacetin with nitric acid and Brockelt<sup>200</sup> applied this procedure to the nitration of acetaminophen. This involved nitration of an aqueous solution of acetaminophen with 5N nitric acid at room temperature for 20 minutes. Brockelt found no absorption maximum for the nitrated acetaminophen in the range 380 to 750 mμ. and decided to use the color formed in an assay procedure measuring the light absorption at 428 mμ. (mid-point of the straight part of the light absorption curve).

Feigl<sup>187</sup> and also Rosenthaler<sup>226</sup> reported that amides such as acetaminophen undergo nitrosylation with nitrous acid to yield an N-nitroso compound which may be saponified to give a diazonium compound suitable for phenolic coupling. Koen<sup>5</sup> noted that in acidic medium with sodium nitrite, acetaminophen gives a yellow color which changes to an orange color on rendering alkaline and uses this color to quantitatively determine acetaminophen. Le Perdriel et al.<sup>186</sup> found that acetaminophen did not form a nitroso compound on reaction with sodium nitrite and dilute hydrochloric acid but the 2-nitro-4-acetamidophenol described by Girard. They found that this reaction could be used as the basis of a colorimetric assay procedure. The solution containing nitrated acetaminophen is made alkaline with sodium carbonate solution and the light absorption measured at the absorption maximum occurring between 440 and 445 mμ.

Inamdar and Kaji<sup>227</sup> employ a similar procedure but do not make the final solution alkaline. This yields a solution of nitrated acetaminophen giving absorption maxima at 375 and 395 mμ., the latter wavelength being used for quantitative measurement.

Hanegraaff and Chastagner<sup>228</sup> continued the work of Le Perdriel<sup>186</sup> studying the mechanism of the nitration of acetaminophen and modified the spectrophotometric assay procedure.

They greatly increased the concentration of acid employing a very strong mixture of nitric and sulphuric acids in addition to the use of sodium nitrite and measured the extinction at 375 mμ.

The method of Le Perdriel has been thoroughly evaluated by Chafetz et al.<sup>229</sup> who have attempted to optimize the reaction parameters and claim the procedure has an excellent precision and is well suited to automated techniques<sup>442</sup>. This procedure<sup>229</sup> is claimed to be specific for the determination of acetaminophen in the presence of a range of excipient materials and drug substances commonly found in formulations containing acetaminophen.

The p-nitrobenzoyl esters of acetaminophen were prepared by Tingle and Williams<sup>35</sup> and by Reverdin and Cuisinier<sup>183</sup> in 1906. More recently the absorption spectra of the 4-nitrobenzoic acid<sup>230</sup> and the 2,4- and 3,5-dinitrobenzoic<sup>231</sup> acid esters of acetaminophen (as well as the trans-4-nitrocinnamic acid ester<sup>232</sup>) have been thoroughly studied and may represent useful colorimetric reagents for the determination of acetaminophen.

Oxidative reactions have been used in the determination of acetaminophen. Basu<sup>250</sup> hydrolysed acetaminophen with hydrochloric acid to give p-aminophenol which was then oxidised with 0.1N potassium dichromate to give a violet coloured oxidation product. This dye was quantitatively extracted into isobutyl alcohol and its absorbance measured spectrophotometrically at 550 mμ.

Brodie and Axelrod<sup>92</sup> found that p-aminophenol from the acid hydrolysis of acetaminophen could be oxidised with sodium hypobromite and the oxidation product coupled with phenol to form an indophenol dye (absorption maximum 620 to 630 mμ.) This procedure has been used to determine acetaminophen in biological material

92,251 and has also been used to determine the level of free p-aminophenol in acetaminophen<sup>62, 203,252</sup>.

The Brodie-Axelrod procedure<sup>92</sup> requires the neutralisation of an acid solution of p-aminophenol with alkali and should the neutralisation point be passed to give an excess of alkali, degradation of the p-aminophenol results. This problem has been overcome by Murfin and Wragg<sup>253, 254</sup> who have been able to remove the need for preliminary hydrolysis of the acetaminophen to p-aminophenol. In their manual procedure<sup>254</sup> the acetaminophen solution is added to a hydrochloric acid-sodium hypochlorite mixture (pH 3.4; ca. 0.25% available chlorine) and the excess hypochlorite is removed with sodium arsenite. The quinone chlorimide thus formed is then coupled with phenol in the presence of a borate buffer (pH 9.9) to give a stable blue indophenol dye ( $\lambda$  max. 625 m $\mu$ .). The procedure yields results with good precision, the mean relative standard deviation obtained by the authors being 0.36%.

Ninomiya<sup>255</sup> oxidised acetaminophen directly with potassium ferricyanide in sodium hydroxide solution at 0°C and then formed an indophenol dye ( $\lambda$  max. 635 m $\mu$ .) by coupling with phenol. The coloured product was tentatively identified by Ninomiya as N-(p-hydroxyphenyl)-p-benzoquinone imine.

Sakurai and Umeda<sup>256</sup> oxidised acetaminophen with chloramine T in the presence of 2,4-dinitrophenyl hydrazine to give a coloured p-benzoquinone imine 2,4-dinitrophenylhydrazone. The color produced can probably be used in a similar manner to that described in a further paper by Umeda<sup>257</sup> which describes the oxidation of acetaminophen with ceric ammonium sulphate in acidified ethanolic solution and is subsequently reacted with 3-methyl-2-benzothiazoline hydrazone. This reaction mixture on neutralisation with tri-

ethanolamine yields a blue violet color ( $\lambda$  max. 580 m $\mu$ .) which facilitates quantitative spectrophotometric measurement.

Routh et al.<sup>206</sup> employed a stable free radical, diphenylpicrylhydrazyl, to abstract a hydrogen atom from acetaminophen (in ethylene dichloride solution) thereby promoting a process of radical coupling. This results in a reduction of the violet color of the diphenylpicrylhydrazyl ( $\lambda$  max. 527 m $\mu$ .) with the formation of yellow diphenylpicrylhydrazine. The decrease in the intensity of the violet color is used to measure the concentration of acetaminophen.

Dedicoat and Symonds<sup>443</sup> found that in a pH 8.0 borate buffer acetaminophen reduces Folin and Ciocalteu's reagent to give a stable blue color ( $\lambda$  max. 700 m $\mu$ .). This was best produced by heating the reaction mixture at 100°C for 10 minutes and could be used to determine acetaminophen in the presence of several other analgesic drugs.

A number of procedures are based on the acid hydrolysis of acetaminophen to p-aminophenol which is then coupled with a suitable agent. A much used procedure employs the reaction of the p-aminophenol with  $\alpha$  - naphthol in alkaline solution, extraction of the coloured reaction product into n-butanol followed by measurement of the extinction at 635 m $\mu$ . This procedure is based on the work of Greenberg and Lester<sup>123,124</sup> and the later papers of Koshy and Lach<sup>209</sup> and Gwilt, Robertson and McChesney<sup>233</sup>, the latter authors employing an  $\alpha$  - naphthol reagent containing potassium dichromate.

Brodie and Axelrod<sup>91,92</sup> modified the procedure and diazotised the p-aminophenol before reacting it with alkaline  $\alpha$  - naphthol to give a dye exhibiting an  $\lambda$  max. at 510 m $\mu$ . This procedure was also employed by Carlo et al.<sup>234</sup> after slight modification.

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Kos<sup>235</sup> reacted the p-aminophenol (obtained by acid hydrolysis of acetaminophen) with alkaline  $\beta$  - naphthol (without diazotisation) to give a green color having an absorption maximum at 420 m $\mu$ .

Mouton and Mason<sup>236</sup> used trichloroacetic acid to hydrolyse acetaminophen to p-aminophenol which they then diazotised and coupled with N<sup>1</sup>-diethyl-N-1-naphthyl-propylenediamine. The dye formed was extracted into amyl alcohol and the extinction determined at 590 m $\mu$ . This procedure was modified slightly by Heirwegh and Fevery<sup>237</sup> who substituted N-(1-naphthyl)ethylenediamine as coupling agent ( $\lambda$  max. 596m $\mu$ ). Ivashkiv<sup>238</sup> has also examined this procedure critically, finding at least 4 hours incubation at room temperature is required for complete color development.

Other procedures have been described where the p-aminophenol obtained has been coupled with alkaline anisaldehyde<sup>239</sup>, acid vanillin<sup>240</sup> and other reagents<sup>241,247</sup>.

It is possible to couple diazotised reagents directly with acetaminophen but they react only slowly<sup>79,105</sup>. Dobáš, Štěrba and Večeřa<sup>79,242</sup> have studied the kinetics of these reactions and propose a reaction mechanism.

Acetaminophen has also been shown to couple with the diazotised reagents shown in Table 4 thus presenting the opportunity for possible spectrophotometric measurement.

Kondo et al.<sup>246</sup> have examined the absorption spectra (and bathochromic shift) produced by the reaction of acetaminophen in alkaline methanolic solution with an p-nitrobenzene diazonium fluoroborate reagent. Acetaminophen has been shown to produce colors of potential spectrophotometric use by interaction with 1-nitroso-2-naphthol, 2-nitroso-1-naphthol and

disodium-3-hydroxy-4-nitroso-2,7-naphthalenedi-sulphonate 248,249.

TABLE 4  
Diazotised Reagents Capable  
of Coupling with Acetaminophen

Reagent	Color of Product	Ref.
Diazotised aniline (acid)	Yellow	36
Diazotised 1,4-Naphthyl-amino-sulphonic acid (alkali)	Red	243
Diazotised 2-Naphthyl-amino-8-sulphonic acid (alkali)	Yellow-brown	243
Diazotised 1,4-Amido-aceto-naphthalide-6-sulphonic acid (alkali)	Red	243
Diazotised 4-Nitro-6-chloro-2-aminophenol	Olive brown	244
Diazotised 4,6-Dinitro-2-aminophenol	-	245

#### 6.25 Ion-Exchange Chromatographic Procedures

Ion-exchange column chromatography has been used to separate acetaminophen from its decomposition product, p-aminophenol, from mixtures containing other medicinal agents and from dosage forms (see Table 5).

Street and Niyogi<sup>211,213</sup> separated acetaminophen from phenacetin (acetophenetidin), phenobarbitone and salicylic acid by two dimensional chromatographic development on diethyl-aminoethylcellulose ion-exchange paper (Whatman DE 20). Initial separation was by simple development in a 0.2N ammonium hydroxide solvent. This was followed by ionophoretic development at right angles in the same solvent (5mA; 250V.).

TABLE 5  
Ion-Exchange Chromatographic Separation of Acetaminophen

<u>Separation from</u>	<u>Ion-Exchange medium</u>	<u>Elutrient</u>	<u>Quantitation</u>	<u>Reference</u>
Elixir formulation	Dowex 1 - X8, 200-400 mesh (hydroxide form)	20% glacial acetic acid in ethanol	Titrimetry in DMF solution with sodium methoxide	196
Phenobarbitone	Dowex 1 - X1	70% methanol	U.V. Spectro- photometry (249mμ.)	207
[Caffeine Salicylamide]	[Dowex 1 - X1 Dowex 50 - X2]	0.1N HCl in 70% ethanol	Differential U.V. Spectrophotometry	205
p-Aminophenol	Amberlite IR-120	Water	U.V. Spectrophoto- metry (244 mμ.)	208
[p-Aminophenol Chlorobenzoxazoline Sulphadimethoxine Caffeine Theophorin Tartrate]	Amberlite IR-120	Water	U.V. Spectrophoto- metry (244 mμ.)	209
[Codeine Phosphate Phenylephrine HCl Pyrilamine Maleate]	Cation Exchange Resin, Alginic acid, Mesh 40-100 (B.D.H.)	Water	U.V. Spectrophoto- metry (249 mμ.)	210

## 6.26 Partition Chromatographic Procedures

Koshy<sup>213</sup> separated acetaminophen from admixture with caffeine and aspirin by partition chromatography employing consecutive sulphuric acid and sodium bicarbonate impregnated Celite columns. Caffeine was retained on the acid column and aspirin on the alkaline column. Acetaminophen which is not ionised under either set of conditions passed through the columns in the diethyl ether solvent.

Levine and Hohmann<sup>214</sup> noted that the above system was unable to separate acetaminophen from neutral or weakly acidic compounds. To overcome this weakness they replaced the sulphuric acid by hydrochloric acid and the sodium bicarbonate by a sodium carbonate-sodium bicarbonate buffer (pH 10.1). The sample containing acetaminophen is incorporated into the acid impregnated Celite before it is packed into the first column which is placed directly above the second alkaline column.

The two columns are washed with chloroform and then the acetaminophen is eluted with ether. The acetaminophen is determined spectrophotometrically in acid methanol solution (249m $\mu$ .) following evaporation of the ether. This procedure will successfully allow the determination of acetaminophen in the presence of many co-administered drug substances.

This procedure<sup>214</sup> was slightly modified by Hohmann<sup>215</sup> who contained the two packing materials in a single column as separated segments. The modified procedure shown to be satisfactory for the determination of acetaminophen in liquid preparations was adopted by the NFXIII for the determination of acetaminophen in Acetaminophen Elixir.

Both procedures<sup>214,215</sup> have been



successfully applied<sup>216</sup> to <sup>219</sup> to the determination of acetaminophen in other formulated products. Further evaluation<sup>220</sup> of the single column procedure indicated that optimum results were obtained if 1N hydrochloric acid and 0.5% ethanol in chloroform are used in place of the concentrated hydrochloric acid and chloroform.

Hamilton<sup>221</sup> has described a further modification to the two-column procedure in which the columns are separated after the chloroform wash and acetaminophen is eluted from the acid column with water-washed ethyl acetate.

Koshy et al.<sup>180</sup> have used a three column system (based on the Levine<sup>222</sup> system) to separate diacetyl-p-aminophenol from tablet formulations containing acetaminophen, aspirin and caffeine.

#### 6.27 Paper and Thin Layer Chromatographic Procedures

A number of thin layer and paper chromatographic methods have been found suitable for the isolation and identification of acetaminophen. The qualitative aspects of these methods are summarised in Tables 6 to 11.

Reversed phase chromatography was used by Tomlinson<sup>8</sup> in a study designed to correlate R<sub>f</sub> characteristics with chemical structure for a series of substituted acetanilides including acetaminophen. In this study<sup>8</sup> two separate stationary phases were used on silica gel G plates.

<u>Stationary Phase</u>	<u>Mobile Phase</u>	<u>R<sub>f</sub> at 20 ± 0.5°C (av. of 10 results)</u>
1-octanol	acetone/water (1:9)	0.758 (0.740 to 0.779)
liquid paraffin (B.P.)	acetone/water (2:8)	0.713 (0.710 to 0.716)

Semi-quantitative procedures relying on the visual comparison of sample spot size and intensity with standards have been described by Klutch and Bordun<sup>131</sup> and by Shand<sup>267</sup>. Büch et al.<sup>270</sup> described a quantitative procedure in which the acetaminophen is acid hydrolysed to p-aminophenol which is then separated (10-100µg./spot) by thin layer chromatography on a Silica Gel G layer. The p-aminophenol is eluted with 0.5N sodium hydroxide solution and determined spectrophotometrically at 240 mµ. A further paper by the same authors<sup>263</sup> employs chromatographic separation of the acetaminophen (without prior hydrolysis) on a layer of Silica Gel GF followed by elution with methanol and spectrophotometric determination at 245 mµ. The procedure may be used to determine acetaminophen (60-300µg./spot) in serum with a precision of ± 5%. Cummings, King and Martin<sup>265</sup> describe a similar quantitative procedure that employs elution of the acetaminophen from the silica gel with water rather than with methanol.

#### 6.28 Vapor Phase Chromatographic Procedures (G.L.C., V.P.C.)

Acetaminophen presents difficulties for quantitative G.L.C. determination as a result of the pronounced elution peak tailing caused by its polar hydroxyl group.

Koibuchi et al.<sup>278</sup> overcame this problem by acetylating the hydroxyl group to give N,O-diacetyl-p-aminophenol (DAPAP) which gave a good symmetrical peak after elution from a 1%

TABLE 6  
Paper Chromatographic Systems for Acetaminophen

<u>Paper</u>	<u>Direction</u>	<u>Solvent System</u>	<u>R<sub>f</sub> Value</u>	<u>Use</u>	<u>Reference</u>
Whatman 3MM	Two dimensional development, ascending	a) Isopropanol/aq. ammonia/water (8:1:1)	0.83	Characteris- ation in human urine	481
		b) Benzene/propionic acid/water (1000:700:41)	0.24		
Whatman No.1	ascending	Water	0.83	Chromatographic study of	188
Whatman No.1	"	Mineral spirits saturated with water	0.00	isomeric phenols	"
Whatman No.1	"	Toluene saturated with water	0.00	" "	"
Whatman No.1 impregnated with tributyrin	ascending	Phosphate buffer (pH 7.4)	0.80	Identity test	3
Whatman No. 1	ascending	Benzene/gl.acetic acid/water/n-butanol (38:38:17:7)	n.a.	Isolation from microbial cul- cultures	181
Grade FN1 (VEB Spezial-papierfabrik)	ascending	n-Butanol/10% aq. ammonia (2:1)	0.61	Separation from other analgesics	2
Grade FN1 impregnated with 4% sodium bi-carbonate (pH9)	ascending	Benzene/acetic acid/water (4:4:2)	0.04	Separation from other analgesics	2

TABLE 7

Thin Layer Chromatographic Systems for Acetaminophen (Neutral Systems)

<u>Absorbent</u>	<u>Solvent System</u>	<u>R<sub>f</sub> Value</u>	<u>Use</u>	<u>Reference</u>
Silica Gel GF	Methanol	0.50	Separation from phenazone	259
Silica Gel G	Methyl ethyl ketone	0.50	Separation from chlorpromazine	260
Silica Gel GF	Methyl ethyl ketone	0.70	Separation from phenazone	259
Silica Gel HF	Butanone	0.44	Identity test	268
Silica Gel GF	Chloroform/diethyl ether (85:15)	0.00	Separation from phenazone	259
Silica Gel G	Chloroform/acetone (90:10)	0.09	Separation from butobarbitone	191
Silica Gel G	Chloroform/methanol (80:20)	0.75	Identity test	261
Silica Gel G	Benzene/acetone (20:10)	0.33	Separation from other analgesic metabolites	262
Silica Gel G	Chloroform/benzene/acetone (65:10:25)	0.33	as above	189
Silica Gel GF	Acetone/n-butanol/water (50:40:10)	0.92	Determination of acetaminophen and metabolites in serum	263
Silica Gel G	<u>Two dimensional development</u>			
	a) Chloroform/acetone (90:10)	n.a.	Determination of acetaminophen and metabolites in urine	264
	b) Chloroform/benzene/acetone (65:5:30)	n.a.		

TABLE 8

Thin Layer Chromatographic Systems for Acetaminophen (Alkaline Systems)

<u>Adsorbent</u>	<u>Solvent System</u>	<u>R<sub>f</sub> Value</u>	<u>Use</u>	<u>Reference</u>
Silica Gel GF	Chloroform/95% methanol/ammonia (85:15:1)	0.47	Isolation from microbial cultures	181
Silica Gel GF	Chloroform/iso-propanol/35% aq. ammonia (45:45:10)	0.80	Determination of acetaminophen and metabolites in serum	263
Silica Gel GF	Chloroform/iso-propanol/33% aq. ammonia (80:15:5)	-		
	lower phase/methanol (90:5)	0.79	as above	263
Silica Gel G	Butylacetate/acetone/n-butanol/10% aq. ammonia (50:40:30:10:)	0.67	Identity test	269
Silica Gel GF	Cyclohexane/chloroform/pyridine (20:60:5)	0.05	Separation from phenazone	259
		0.06	Identity test	268

TABLE 9  
Thin Layer Chromatographic Systems for Acetaminophen (Acidic Systems)

	<u>Adsorbent</u>	<u>Solvent System</u>	<u>R<sub>f</sub> Value</u>	<u>Use</u>	<u>Reference</u>
	Silica Gel G	Chloroform/ethanol/acetic acid (88:10:2)	Chromatogram diagram	Separation from DAPAP and other analgesics	121
	Silica Gel G	Chloroform/acetone/acetic acid (80:18:2)	as above	as above	121,180
	Silica Gel GF	Benzene/methanol/acetic acid (45:8:4)	0.58	Determination of acetaminophen and metabolites in serum	263
	Silica Gel GF	Ethyl acetate/methanol/water/acetic acid (60:30:9:1)	0.82	Determination of acetaminophen and metabolites in urine	265,266
2	Silica Gel GF	Double development a) Benzene/diethyl ether/acetic acid/methanol (120:60:18:1) b) Ethyl acetate/diethyl ether (80:20)	Chromatogram illustrated	Separation from DAPAP and other analgesics	120
	Brinkman Al oxide GF	Toluene/benzene/water/acetic acid (2:2:1:2) (upper phase)	0.10	Separation from other metabolites	154
	Brinkman Al oxide GF	Chloroform/methanol/water/acetic acid (20:10:20:1) (lower phase)	0.35	as above	154
	Brinkman Al oxide GF	Cyclohexane/n-propanol/water/acetic acid (20:20:20:1) (upper phase)	0.84	Separation of phenacetin metabolites	131

cont'd.....

TABLE 9 (cont'd)  
Thin Layer Chromatographic Systems for Acetaminophen (Acidic Systems)

<u>Adsorbent</u>	<u>Solvent System</u>	<u>R<sub>f</sub> Value</u>	<u>Use</u>	<u>Reference</u>
Brinkman Al oxide GF	Ethylene dichloride/methanol/ water/acetic acid (20:10:20:1) (lower phase)	0.40	Separation of phenacetin metabolites	131
Brinkman Al oxide GF	Ethylene dichloride/methanol/ water/acetic acid (30:5:10:3)	0.16	as above	131
Silica Gel G	Butyl acetate/chloroform/ 85% formic acid (60:40:20)	0.46	Identity test	269
Silica Gel GF	Dichloroethane/ethyl acetate/ 98% formic acid (60:20:20)	0.65	as above	263

TABLE 10  
Reagents for Paper Chromatographic Visualisation of Acetaminophen

<u>Reagent</u>	<u>Color</u>	<u>Sensitivity</u> ( $\mu$ g. acetaminophen)	<u>Reference</u>
1. U.V. - Fluorescence	Blue-Grey	20	2
2. U.V. - Fluorescence after spraying with 0.5% ethanolic oxine	Yellow	1	2
3. Diazotised p-nitroaniline	Violet	-	2,258
4. 5% ethanolic ferric chloride	Violet	-	2,181
5. 15% aq. ferric chloride/ 1% aq. potassium ferricyanide (1:1)	Deep blue	< 1	2
6. Ferric chloride/potassium ferricyanide/Millon's reagent (2:2:2)	Violet	20	2
7. Ammoniacal silver nitrate (0.1N)	Black	-	2
8. Bromine/starch/potassium iodide	Blue	-	3
9. Ceric ammonium nitrate	Purple	-	188



TABLE 11  
Reagents for Thin Layer Chromatographic Visualisation of Acetaminophen

<u>Reagent</u>	<u>Color</u>	<u>Sensitivity</u> ( $\mu\text{g. acetaminophen}$ )	<u>Reference</u>
1. U.V. (254m $\mu$ .) - fluorescence quenching	Dark spot	0.5	267
2. 5% aq. silver nitrate	Black	0.25-0.5	259, 262, 267
3. 10% aq. silver nitrate	Black	0.2	189, 264
4. 10% ferric chloride and 0.5% potassium ferricyanide in water	Dark blue	0.1	181, 267
5. 5% aq. ferric chloride	Grey blue	< 5	189
6. Folin and Ciocalteu reagent	Blue	0.5	263
7. p-Dimethylaminobenzaldehyde/ hydrochloric acid	Yellow	1	189
8. Iodine vapor	n.a.	n.a.	180
9. Pauly reagent	n.a.	n.a.	131
10. Diazotised o-dianisidine	n.a.	n.a.	131
11. Diazotised sulphanilic acid	n.a.	n.a.	120

DEGS column. The acetaminophen was acetylated with a pyridine-acetic anhydride reagent employing strictly controlled reaction conditions to suppress the formation of N,N,O-triacetyl-p-aminophenol (TAPAP), a secondary product of the reaction. Quantitation was effected by peak height ratio measurement using an internal standard and almost theoretical recoveries were obtained from laboratory prepared mixtures (standard deviation 0.4 to 0.5%).

Prescott<sup>277</sup> has more recently employed a similar procedure for the determination of acetaminophen in plasma (standard deviation about 3.5%).

Acetaminophen may be readily silylated to form derivatives suitable for quantitative G.L.C. determination<sup>279,280,281,292</sup>. Prescott<sup>280</sup> used a N-trimethylsilylimidazole (TMSI) reagent to selectively silylate the hydroxyl group. In a separate procedure he<sup>280</sup> used a N,O-bis (trimethylsilyl) acetamide (BSA) reagent which produces a di-TMS derivative by silylating both the hydroxyl group and the amide nitrogen. He reports<sup>280</sup> near theoretical recoveries (TMSI procedure) for acetaminophen from plasma and urine with standard deviations of 1.8 and 2.8% respectively (calculation from peak height ratio with an internal standard).

The direct G.L.C. determination of acetaminophen has been described<sup>272,274,275</sup> but the accuracy seems generally to be inferior to that of the indirect procedures and the working range for sample size is narrower as a result of poor peak symmetry.

Table 12 gives details of the various G.L.C. systems described for the separation, identification and quantitative determination of acetaminophen.

TABLE 12  
G.L.C. (V.P.C.) Determination of Acetaminophen

Column Support	Column Stationary Phase	Column Temp.	Retention Time	Detector System	Internal Standard	Type of Determination	Reference
Chromosorb W (AW-DMCS) 80/100 (mesh)	10% UCC-W-982	195°C	ca.1 min.	F.I.D.	Amitriptyline hydrochloride	Analgesic Preparations	272
Anakrom AS 80/90	1% SE-30 plus 1% Carbowax 20M	200°C	3 min.	Electron Capture (Sr-90)	External Standard	Metabolic	154
6% QF-1-0065 60/70		160°C	ca.3 min.	Electron Capture (Tritium Foil)	-	Qualitative-Clinical	258
Chromosorb W (AW)	2% SE-30 plus 0.1% Tristerarin	180°C	3.5min.	F.I.D.	-	Toxicological	273
Gas Chrom Q 100/120	3% OV-17	165°C	ca.5 min.	KCl-T.I.D.	Amobarbital	Pharmaceutical Preparations	274
Aeropak 30 70/80 (in silanised column)	2% FFAP	240°C	6.5 min.	F.I.D.	Diphenyl phthalate	Metabolic	275
Anakrom AS 80/90	0.5% SE-30 plus 0.5% Carbowax 20M	190°C	10 mins.	Electron Capture (Sr-90)	External Standard	Metabolic	131

TABLE 12 (cont'd)  
G.L.C. (V.P.C.) Determination of Acetaminophen

<u>Column Support</u>	<u>Column Stationary Phase</u>	<u>Column Temp.</u>	<u>Retention Time</u>	<u>Detector System</u>	<u>Internal Standard</u>	<u>Type of Determination</u>	<u>Reference</u>
Gas Chrom Q 80/100	1% HI-EFF-8BP plus 10% SE-52	220°C	ca.17 min.	F.I.D.	External Standard	Pharmaceutical Preparations	182
Chromosorb W (AW-HMDS)	10% Apiezon L	210°C	2.4 relative to barbitone	F.I.D.	-	Qualitative	3
Chromosorb W 60/80 (silanised)	5% SE-30 or 3% Neopentyl glycol succinate polyester	190°C or 200°C	n.a.	n.a.	n.a.	Pharmaceutical Preparations	276
Chromosorb G 70/80	5% Carbowax 20M	225°C	n.a.	F.I.D.	External Standard	Analgesic mixtures	180
Gas Chrom Q 100/120	3% HI-EFF-8BP	220°C	3.4min. (as O-acetyl acetaminophen)	F.I.D.	N-butyl-p-aminophenol (as O-acetyl deriv.)	Metabolic	277
Chromosorb W 60/80 (AW-silanised)	1% Diethylene-glycol succinate polyester	180°C	ca.8 min. (as O-acetyl acetaminophen)	F.I.D.	N,O-diisobutyl-p-aminophenol	Antipyretic Preparations	278
Gas Chrom Q 100/120	5% Apiezon L	160°C	9 min. (as T.M.S. ether of acetaminophen)	F.I.D.	n-Hexadecane	Pharmaceutical Preparations	279

TABLE 12 (cont'd)  
G.L.C. (V.P.C.) Determination of Acetaminophen

Column Support	Column Stationary Phase	Column Temp.	Retention Time	Detector System	Internal Standard	Type of Determination	Ref.
Gas Chrom Q 80/100	5% OV-1	155°C	ca.15.6 min. (as B.S.A.* deriv.)	F.I.D.	p-Bromo-acetanilide (as B.S.A.* deriv.)	Metabolic	280
Gas Chrom Q 80/100	10% OV-17	200°C	ca.17.6 min. (as T.M.S.I.**)	F.I.D.	p-Chloro-acetanilide (as T.M.S.I.** deriv.)	Metabolic	280
Chromosorb W (HP) 80/100	5% OV-101	Programmed from 100 to 300°C at 10 deg./min.	10.9 min. (as B.S.T.F.A.*** deriv.)	F.I.D.	-	Qualitative Pathological	11
Gas Chrom Q	3% OV-1	160°C	n.a. (as B.S.T.F.A.*** deriv.)	F.I.D.	p-Bromo-acetanilide (as B.S.T.F.A.*** deriv.)	Metabolic	281
Gas Chrom Q	OV-17	190°C	3.3 min. (as H.M.D.S.+ deriv.)	F.I.D.	Docosane (as H.M.D.S.+ deriv.)	Metabolic	447

- \* B.S.A. - N,O-bis (trimethylsilyl) acetamide
- \*\* T.M.S.I. - N-trimethylsilylimidazole
- \*\*\* B.S.T.F.A. - bis (trimethylsilyl) trifluoroacetamide (Regisil)
- + H.M.D.S. - hexamethyl disilazane/trichloromethyl silane

#### 6.29 High-Pressure Liquid Chromatographic and Gel Filtration Procedures

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Burtis, Butts and Rainey<sup>11</sup> first described the use of high-pressure liquid chromatography in the determination of acetaminophen and its glucuronide metabolite in urine. Their procedure employed a high-pressure anion exchange chromatographic system<sup>293</sup> with a U.V. detector and gave very long retention times in excess of 16 hours.

Anders and Latorre<sup>295</sup> have more recently developed an improved procedure capable of resolving acetaminophen and its glucuronide and sulphate conjugates present in urine within a total elution time of 40 min.

Henry and Schmit<sup>294</sup> described a rapid high-pressure anion exchange chromatographic procedure for the determination of acetaminophen in analgesic tablets using a peak area ratio measurement with an internal standard. A plot of peak area versus concentration was linear for both acetaminophen and the salicylamide<sub>3</sub> internal standard over a dynamic range of  $5 \times 10^3$ . This gave a possible range for acetaminophen determination of 3 mg. to about 50  $\mu$ g. per sample injection.

Stevenson and Burtis<sup>296</sup> have improved on this procedure and describe a rapid high-pressure liquid chromatographic assay for acetaminophen in a wide range of analgesic tablets claiming a precision giving a relative % standard

deviation of 0.79 (using an external standard). Details of the procedures are given in Table 13.

Jagenburg, Nagy and Rödger<sup>297</sup> separated the conjugated metabolites of acetaminophen by a gel filtration technique using Sephadex G-10 (Pharmacia) but no mention is made of the elution of acetaminophen.

Brook and Munday<sup>89</sup> studied the interaction of a series of compounds including acetaminophen with Sephadex G-10 and Sephadex LH-20 eluting with 0.1N sodium hydroxide solution.

### 6.3 Automated Procedures

Ederma et al.<sup>282</sup> automated the Brodie and Axelrod<sup>92</sup> procedure for the determination of acetaminophen in serum.

The ether extraction of acetaminophen from the serum and its backwashing into dilute caustic soda remained as manual procedures but the conversion of the acetaminophen to p-aminophenol and the colorimetric determination were automated using an Auto Analyser system. A later paper<sup>283</sup> describes the application of basically the same system to the determination of acetaminophen in whole blood.

Shane and Kowblansky<sup>78</sup> automated their differential U.V. spectrophotometric procedure (see Section 6.23) for the determination of acetaminophen in the presence of aspirin, salicylamide and caffeine. Alber and Overton<sup>274</sup> also determining acetaminophen in the presence of salicylamide and caffeine used a G.L.C. system with automated peak height measurement and calculation. This system employed an amplified KCl thermionic detector system with a direct feed into the analog-to-digital converter of a PDP 12A LINC System computer. It is suggested that the sample preparation could also be automated.

Daley, Moran and Chafetz<sup>442</sup> automated

TABLE 13

High-Pressure Liquid Chromatographic Determination of Acetaminophen

<u>Instrument</u>	<u>Column Size and Packing</u>	<u>Column Temp. and Pressure</u>	<u>Flow Rate (ml./hr.)</u>	<u>Elution</u>	<u>Retention Time</u>	<u>Ref.</u>
"UV-ANALYSER" (Oak Ridge National Lab- oratory) with Photometer Detector (260 and 290m $\mu$ .)	0.45x200cm. Dowex 1-X8 (5 to 10 $\mu$ )	25°C increasing to 60°C after 16 hr. 1000-2000 p.s.i.g.	30	Ammonium Chlo- ride-Acetic Acid Buffer pH 4.4 0.015M(380ml.) 1.0M (370ml.) 4.0M (360ml.) 6.0M (525ml.)	Acetaminophen 16.5 hr. Acetaminophen glucuronide 22.4 hr.	11, 293
Du Pont Model 820 Liquid Chromatograph with Model 410 Photometer Detector (254m $\mu$ .)	0.21x1000cm. Zipax coated with strong anion ex- change resin	Temperature n.a. 1200 p.s.i.g.	90	Buffer (Fisher Gram-Pac) pH 9.2 containing 0.005M ammonium nitrate	ca. 2 mins.	294

(cont'd.....)



TABLE 13 (cont'd)  
High-Pressure Liquid Chromatographic Determination of Acetaminophen

<u>Instrument</u>	<u>Column Size and Packing</u>	<u>Column Temp. and Pressure</u>	<u>Flow Rate</u> (ml./hr.)	<u>Elution</u>	<u>Retention Time</u>	<u>Ref.</u>
Varian LCS-1000 with Photometer Detector (254 mμ.)	0.10x250cm. LSF pellicular anion ex- change resin	80°C 800-1000 p.s.i.g.	30	10.0mM formic acid (pH3) con- taining 1.0M potassium chlor- ide (gradient system also given)	Acetaminophen 3.6 min. Acetaminophen glucuronide 2.7 min. Acetaminophen sulphate 9.5 min.	295
Varian LCS-1000 with Photometer Detector (254 mμ.)	0.10x300cm. LSF pellicular anion exchange resin	60°C 925-1000 p.s.i.g.	8.6	1.0M Tris Buffer (pH 9.0)	Acetaminophen 649 secs. (+ 1.08%)	296

the colorimetric procedure of Chafetz et al.<sup>229</sup> (see Section 6.24).

Murfin<sup>253</sup> has automated a colorimetric procedure based on the chromogenic reaction of acetaminophen with an acid hypochlorite - alkaline phenol reagent system.

The procedure which may be used for single tablet assay of acetaminophen, alone or in combination with aspirin and codeine phosphate is based on a Technicon 25-channel system preceded by a sampling unit and a Technicon continuous filter. The complete procedure from commencement of sampling to the recording of the maximum color takes only 11 mins. and yields results with a coefficient of variation of about 0.4%. The sampling time takes 2 min. 15 secs. followed by a wash time of 45 secs. thus permitting the examination of up to 20 samples per hour on a continuous basis.

#### 6.4 Radiochemical Procedures

Davison et al.<sup>284</sup> described the preparation of N-(1-<sup>14</sup>C-Acetyl)-p-aminophenol from sodium hydrosulphite washed, p-aminophenol and (acetyl <sup>14</sup>C) acetic anhydride giving a product with an activity of 0.88  $\mu$ C/mg. Koss et al.<sup>285</sup> prepared quantities of acetaminophen labelled on the nucleus or acetyl side chain.

N-Acetyl-2,6-<sup>14</sup>C-p-aminophenol was prepared by the reaction of sodium nitromalondialdehyde monohydrate with 1,3-<sup>14</sup>C-acetone to give 2,6-<sup>14</sup>C-p-nitrophenol. This was then reduced and concurrently acetylated to give the required product.

N-(1-<sup>14</sup>C-Acetyl)-p-aminophenol was produced by reacting p-aminophenol in peroxide free tetrahydrofuran with 1-<sup>14</sup>C sodium acetate.

The determination of radioactivity in the organs of Wistar-Rats dosed with either of the labelled compounds was carried out employing

a Packard Scintillation Counter. Samples were dissolved in a benzalkonium chloride solution, decolorised with hydrogen peroxide and a scintillator solution added which contained naphthalene, PPO and POPOP.

Radiolabelled metabolites were estimated after thin layer chromatographic separation using a Berthold T.L.C. Radioactivity Scanner.

#### 6.5 Determination of Trace Impurities and Degradation Products

The impurity profile of acetaminophen has already been discussed (see Section 4.13).

The early compendial procedures for the determination of p-aminophenol in acetaminophen relied on colorimetric limit tests employing either a sodium nitro-prusside reagent<sup>21</sup> or the phenol-hypobromite reaction<sup>62</sup>. The latter procedure was shown<sup>252</sup> to be capable of quantitative use having a precision of about  $\pm 5\%$  for a p-aminophenol level in acetaminophen of 0.012%.

More recently the NF XIII<sup>14,7</sup> has adopted a thin layer chromatographic procedure for the determination of traces ( $\leq 0.025\%$ ) of p-aminophenol in acetaminophen. The procedure uses silica gel (HR grade) plates and a methyl ethyl ketone/acetic acid (9:1) solvent system. Visualisation is achieved with an acid p-dimethylaminocinnamaldehyde spray reagent and the size and intensity of the sample spot is compared with a standard spot.

p-Chloroacetanilide levels in acetaminophen were determined by Savidge and Wragg<sup>119</sup> using a thin layer chromatographic separation which employed a solvent mixture of cyclohexane/acetone/diisobutylketone/methanol/water (100:80:30:5:1). This procedure was designed for a p-chloroacetanilide limit test of  $\leq 0.03\%$ . The NF XIII<sup>14,7</sup> limits the level of p-chloroacetan-

ilide to 10 p.p.m. and describes a thin layer chromatographic procedure (solvent-chloroform/benzene/acetone (65:10:25)) capable of this sensitivity.

Savidge and Wragg<sup>119</sup> showed their T.L.C. procedure to be capable of separating O-acetyl-acetaminophen (DAPAP) from acetaminophen and using this procedure found levels of up to 0.09% DAPAP in commercial samples of acetaminophen.

Several other T.L.C. procedures have been described<sup>120,121,180</sup> for the determination of DAPAP in acetaminophen and quantitative determinations have also been made using a G.L.C. system<sup>180</sup>. (see Section 5.6).

The limitation of the content of quinonimine type oxidation products has been achieved mainly by close control of the white color of acetaminophen.

## 6.6 Determination of Acetaminophen and its Metabolites in Body Fluids and Tissues

### 6.61 Determination in Urine

The majority of the published work centres on the determination of free and conjugated acetaminophen in human and animal urine.

Lester and Greenberg<sup>124</sup> determined the metabolites of acetanilide by colorimetric reaction with  $\alpha$  - naphthol of the p - aminophenol produced after acid hydrolysis. Acetaminophen was selectively determined by the same colorimetric procedure after extraction into ethylene dichloride from urine, salted out with dibasic potassium phosphate.

Smith and Williams<sup>125</sup> examining rabbit urine containing acetanilide metabolites, hydrolysed ether extracted urine by heating with acid thus liberating p-aminophenol from the acetam-

inophen conjugates. The p-aminophenol was determined gravimetrically after isolation.

Brodie and Axelrod<sup>92</sup> determined free acetaminophen in urine by a procedure involving the extraction of the acetaminophen from sodium chloride saturated urine into a solvent mixture of isoamyl alcohol and diethyl-ether. The extracted acetaminophen was then acid hydrolysed to p-aminophenol and determined colorimetrically after diazo coupling with  $\alpha$  - naphthol. Conjugated acetaminophen was calculated by difference from total acetaminophen determined as total p-aminophenol obtained by direct acid hydrolysis of the urine. In this case the p-aminophenol was determined by the phenol/hypobromite colorimetric procedure.

Koshy and Lach<sup>208</sup> modified the Lester and Greenberg<sup>124</sup> procedure separating the p-aminophenol from acid hydrolysed urine on an ion-exchange column prior to colorimetric determination. Several authors<sup>91,126,251,264,298,299</sup> have used slight modifications of the two procedures described by Brodie and Axelrod<sup>92</sup> for the determination of free and conjugated acetaminophen in both human and rabbit urine. Levy and Yamada<sup>300</sup> used the Brodie and Axelrod<sup>92</sup> procedure but deconjugated the metabolites by incubation with an enzyme mixture rather than relying on acid hydrolysis. Heirwegh and Fevery<sup>237</sup> retained the acid hydrolysis procedure for deconjugation of the metabolites but substituted the Bratton-Marshall<sup>301</sup> diazotisation procedure for the diazo coupling with  $\alpha$  - naphthol.

Lower, Murphy and Bryan<sup>302</sup> employed both enzymic hydrolysis and the Bratton-Marshall colorimetric procedure for the assay of acetaminophen glucuronide in urine. In this case the sample preparation involved a preliminary fractionation step on a cation-exchange resin.

A third colorimetric procedure<sup>303</sup> has also been described involving diazo coupling of

p-aminophenol (from acid hydrolysis of acetaminophen and metabolites) with o-cresol.

Welch et al.<sup>304</sup> examining the metabolism of acetaminophen in animals determined the conjugated metabolites in urine (after  $\beta$  - glucuronidase and sulphatase hydrolysis) by a colorimetric procedure involving the formation of ion-pairs of acetaminophen with methyl orange.

Acetaminophen and its conjugated metabolites have been determined in urine after thin-layer chromatographic separation by U.V. spectrophotometry<sup>131,263,265,267,270</sup> and by measurement of radioactivity<sup>285</sup>.

Vapor phase chromatography has been used extensively to measure acetaminophen and its metabolites in urine. Grove<sup>275</sup> determined free acetaminophen in urine by a direct G.L.C. procedure employing an ether extract of treated urine. Klutch and Bordun<sup>131,154</sup> also determined conjugated acetaminophen using a preliminary enzymic ( $\beta$ -glucuronidase) hydrolysis step.

Prescott, Steel and Ferrier<sup>292</sup> describes a procedure for the determination of both free and conjugated acetaminophen in urine. Their procedure requires the formation of the trimethylsilyl derivative of acetaminophen which is then chromatographed. Conjugated metabolites are enzymically hydrolysed to give free acetaminophen suitable for trimethylsilylation. Improvements of this approach have also been described<sup>277,280,281,447</sup>.

High pressure liquid chromatography has been used to determine acetaminophen, acetaminophen glucuronide and acetaminophen sulphate directly without hydrolysis or derivative formation<sup>11,295</sup>. Similarly gel filtration procedures<sup>297</sup> may be used but the chromatographic separation is tedious.

S-(1-acetamido-4-hydroxyphenyl) cysteine and 1-acetamido-4-hydroxyphenylmercapturic acid, minor metabolites of acetaminophen have been determined in urine by a gel filtration procedure<sup>297</sup>. The cysteine compound has also been determined<sup>305</sup> in urine following ion-exchange chromatographic separation by a ninhydrin colorimetric procedure.

#### 6.62. Determination in Serum, Plasma and Whole Blood

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The procedures for the determination of acetaminophen and its metabolites in blood are essentially similar to those described above for its determination in urine.

Lester and Greenberg<sup>124</sup> treated both blood and plasma samples with tungstic acid to precipitate proteins and determined the acetaminophen derivatives using the same procedure as described for urine. Gwilt, Robertson and McChesney<sup>306</sup> described a procedure for the determination of free and total acetaminophen in plasma and in whole blood which is essentially a modification of the Lester and Greenberg<sup>124</sup> procedure. In this procedure whole blood is triturated with anhydrous sodium sulphate to give a dry friable mass from which free acetaminophen is extracted with 1.5% isopentanol in diethyl-ether. Acetaminophen is back washed with alkali, hydrolysed with acid to give p-aminophenol which is coupled with alkaline  $\alpha$ -naphthol as in the Lester and Greenberg procedure. However, the green solution so produced is then saturated with potassium chloride and the chromophore extracted into butanol for spectrophotometric measurement. This is claimed to increase the sensitivity of the procedure  $2\frac{1}{2}$  times.

The Brodie and Axelrod procedures<sup>91, 92, 126, 307</sup> for plasma and serum are essentially as described for urine after suitable sample preparation. These procedures have also been automated<sup>282, 283</sup> for the determination of acetamin-

ophen in blood.

The Heirwegh and Fevery<sup>237</sup> procedure which employs the Bratton-Marshall colorimetric system has been used for determinations in serum as described for determinations in urine. This procedure has also been used by Ivashkiv<sup>308</sup> who critically evaluated the reaction parameters.

Routh et al.<sup>206</sup> employed two procedures for the determination of acetaminophen in serum or plasma, one employing differential U.V. absorption spectrophotometry and the other the decolorisation of diphenylpicrylhydrazyl dye.

Büch, Pfleger and Rudiger<sup>263</sup> determined acetaminophen in serum by a quantitative thin-layer chromatographic procedure, the acetaminophen eluted from the sample spot being quantified by a U.V. spectrophotometric procedure. Koss et al.<sup>285</sup> used quantitative thin layer chromatography to determine radiolabelled acetaminophen and its metabolites in human serum, measurement being made with a radio-autography scanner.

Free acetaminophen has been determined in plasma by vapor phase chromatography by several authors<sup>275,277,280,281,292</sup>. The chromatographic procedures in each case are those described for the determination in urine. The sample preparation however, differs slightly. Grove<sup>275</sup> extracts the acetaminophen into ether from plasma saturated with solid ammonium sulphate. Thomas and Coldwell<sup>281</sup> also extract the acetaminophen into ether but buffer the plasma to pH 7.4 with phosphate buffer and then saturate the solution with sodium chloride.

In all the papers by Prescott and co-workers<sup>277,280,292</sup> the plasma is buffered to pH 7.4 with phosphate buffer and the acetaminophen extracted into ethyl acetate. Amsel and Davison<sup>447</sup> also use extraction into ethyl acetate.



### 6.63 Determination in Tissue and Organs

Brodie and Axelrod<sup>92</sup> determined acetaminophen and total conjugated p-aminophenol in homogenised tissue (emulsified in acid)<sup>309</sup> essentially using the same procedures they described for similar determinations in urine. Gwilt, Robertson and McChesney<sup>306</sup> used a very similar procedure to Brodie and Axelrod homogenising the tissue in 0.1N hydrochloric acid, neutralising and buffering to pH 6.6 before extracting the free acetaminophen.

Davison et al<sup>284</sup> and Koss et al.<sup>285</sup> describe the radioassay of total acetaminophen in tissue and organ homogenates using radiolabelled acetaminophen and also describe the separation of free acetaminophen and the individual conjugates in bile by a radioautographic procedure.

## 7. Metabolic Transformations

### 7.1 Metabolism in Man

#### 7.11 Adults

Lester and Greenberg<sup>124</sup> and Brodie and Axelrod<sup>91,92,126</sup> established that acetaminophen is the main metabolite of acetanilide and acetophenetidin (phenacetin). Thus the main metabolites excreted in the urine after administration of acetanilide, acetophenetidin, buccetin<sup>310</sup> or acetaminophen are the glucuronide and ether sulphate conjugates of acetaminophen<sup>124,125,126</sup>.

Acetaminophen sulphate had already been isolated in 1889 by Mörner<sup>122</sup> from the urine of patients who had received acetanilide. Smith and Williams<sup>125,130</sup> isolated crude acetaminophen glucuronide from rabbit urine and Shibaski et al.<sup>266</sup> purified this isolated material and also produced it synthetically.

Minor metabolites have been identified

by Jagenburg and Toczko<sup>305</sup> and by Jagenburg, Nagy and Rödger<sup>297</sup>. These are the cysteine and mercapturic acid conjugates of acetaminophen. The recent findings of Nery<sup>311</sup> of four new metabolites of acetophenetidin suggest that the list of acetaminophen metabolites may not yet be complete<sup>329</sup>. Focella, Heslin and Teitel<sup>448</sup> identified a metabolite of acetophenetidin isolated from dog urine as 4-hydroxy-3-methylthioacetanilide. This substance may also be a metabolite of acetaminophen. In the same study<sup>444</sup> the S-(1-acetamido-4-hydroxyphenyl) cysteine found by Jagenburg and Toczko<sup>305</sup> was tentatively identified more correctly as 3-[(5-acetamido-2-hydroxyphenyl)thio] alanine.

Burtis et al.<sup>11</sup> described the formation of 3-methoxy-acetaminophen (by a girl with a neuroblastoma) after treatment with acetophenetidin. They<sup>11</sup> ascribe the formation of this metabolite and its excretion as the glucuronide to an induced activity of the hydroxylase and catechol O-methyl transferase enzyme systems caused by the high level of acetaminophen (see also refs. 331 and 332).

The metabolic routes are summarised in Fig. 7.

The relative amounts of free acetaminophen and its sulphate and glucuronide conjugates excreted in the urine vary with the individual. Typical results<sup>265,297,300,312</sup> for a dose of 1 to 2 gm. acetaminophen show 75 to 90% of the dose is excreted in the urine with the acetaminophen and its metabolites distributed (approximately) in the following manner:-

Free acetaminophen 2 to 5% (of total excreted)  
 Acetaminophen glucuronide 55 to 75% (but some results are much lower)  
 Acetaminophen sulphate 20 to 40%  
 Acetaminophen 3-cysteine 0.5 to 7% (only 3 results)  
 Acetaminophen 3-mercapturic acid 5 to 7% (only 3 results).

# ACETAMINOPHEN

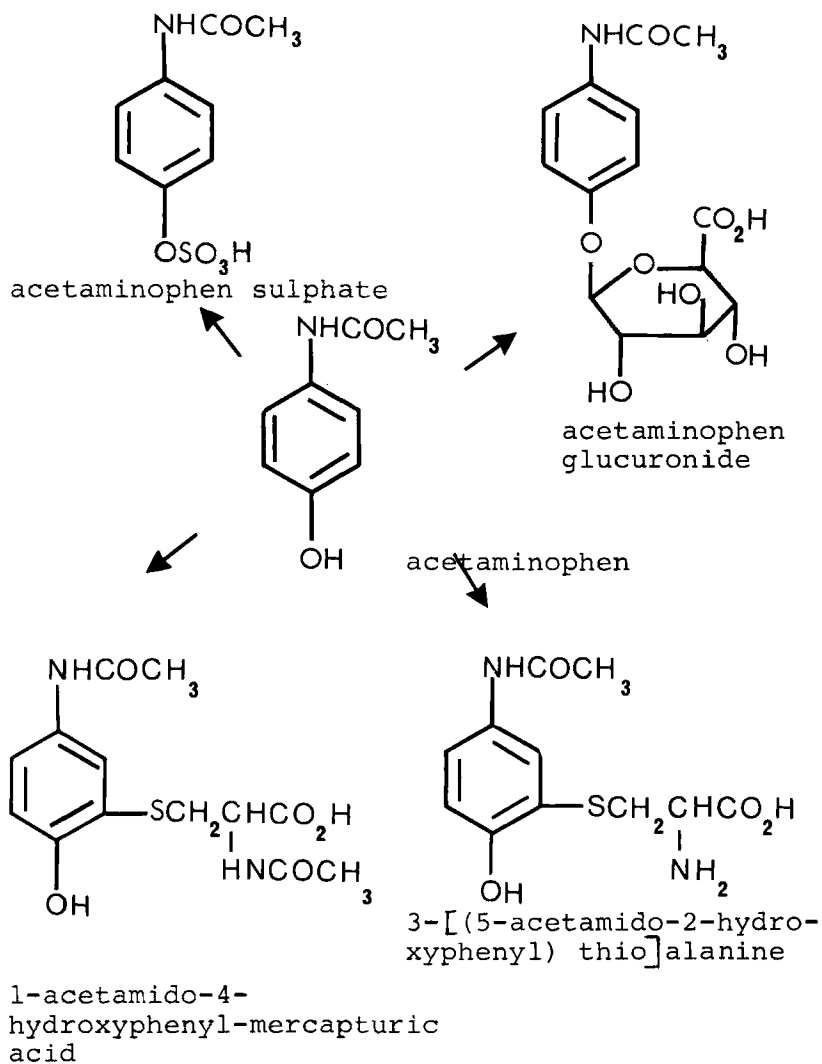


FIGURE 7 - Metabolic Pathways of Acetaminophen

Patients suffering with chronic hepatitis and with liver cirrhosis show a decrease in the blood serum and urine levels of acetaminophen glucuronide and increased levels of free acetaminophen. This results from a corresponding decrease in the activity of glucuronyltransferase in the pathologic livers<sup>237,313,314,315,316,317</sup>. Renal insufficiency does not effect the ratio of free to conjugated acetaminophen in the plasma but through a decrease in glomerular filtration it may increase the plasma level of total acetaminophen by as much as 4-fold<sup>318</sup>. The metabolism of acetaminophen to its sulphate can be blocked by salicylamide which competes with the acetaminophen for sulphate<sup>300,319</sup>. This effect can be counteracted by L-cysteine, a well absorbed source of sulphate<sup>300</sup>. This may be due to sulphate availability being the capacity-limiting factor<sup>300,320</sup>. Salicylamide also decreases the excretion of acetaminophen glucuronide possibly by a similar mechanism. Salicylic acid has no significant effect on the formation of acetaminophen sulphate and glucuronide<sup>312</sup>.

#### 7.12 Newborn Infants

Vest and co-workers<sup>307,323</sup> found that in newborn infants acetaminophen (produced by the administration of acetanilide) is much more slowly conjugated to the glucuronide than in older children and adults. Similar results have been obtained after the administration of acetaminophen<sup>324,325,326,327</sup> and it has been suggested<sup>326,327</sup> that the urinary excretion and blood levels of acetaminophen conjugates depend on the maturity of the glucuronide-forming enzyme system (glucuronic acid transferase and uridine diphosphate glucuronic acid) and the development of renal tubular function.

#### 7.2 Metabolism in Animals

Clark<sup>328</sup> demonstrated that the metabolic pathways of acetaminophen in man and dog were

similar. It has been shown in rats<sup>285</sup> that about 30% of the dose was secreted with the bile in 4 hr. The acetaminophen in the rat bile was shown to be almost completely conjugated to the glucuronide (83%) and the sulphate (14%) and only about 2.5% free acetaminophen was still available.

Acetaminophen metabolism has been studied in the rat<sup>132,263,285,320</sup> cat,<sup>304</sup> dog<sup>304</sup> and rabbit<sup>125,127,330</sup>.

Cats dosed with acetaminophen metabolise the drug in a different manner from man, dog, rabbit and rat, in that less than 6% of the dose was excreted in the urine as acetaminophen glucuronide<sup>304</sup> and less than 2% as acetaminophen sulphate<sup>304</sup>. It has been reported<sup>333,334</sup> that the cat has an impaired ability to form glucuronides, and this defect has been attributed to the lack of the glucuronyl transferase enzyme in the liver.<sup>335</sup> The cat, however, does excrete acetaminophen in the urine, in a conjugated form (capable of enzymic hydrolysis with  $\beta$ -glucuronidase) and it has been suggested by Welch et al.<sup>304</sup> that it may be conjugated with cysteine.

These same authors<sup>304</sup> found that 10 to 13% of the dose administered to cats appears in the urine as an aromatic primary amine but this does not appear to be p-aminophenol.

Acetaminophen is metabolised in the rat and rabbit in a similar manner to that in man but with different ratios of the metabolites<sup>125,127,263,330</sup>.

## 8. Drug Availability

### 8.1 Pharmacokinetics

Many authors have described various aspects of the pharmacokinetics of acetaminophen<sup>68,80,91,124,127,148,234,285,292,298,299,300,306,322,336</sup> to <sup>347</sup>.

Gwilt et al.<sup>336</sup> examined the absorption of acetaminophen in man following oral administration. They<sup>336</sup> found the highest average blood level of total acetaminophen was reached after between 30 and 90 min. depending on the individual. The effects of food and sleep on the absorption and excretion of acetaminophen have been examined by McGilveray and Mattok<sup>454</sup>. Koss et al.<sup>285</sup> followed the administration (100 mg./kg.) of labelled acetaminophen in rats, showing that rapid absorption occurs in the first half hour, only about 40% of the dose reaching the small intestine. This gradually reaches the large intestine where the continued absorption appears to be compensated for by biliary secretion of acetaminophen (about 30% of the dose).

The plasma half lives reported vary as shown in Table 15.

TABLE 15  
Acetaminophen Plasma Half - Life in Man

<u>Author</u>	<u>Plasma half-life (<math>t_{\frac{1}{2}}</math>)</u> (hours)	<u>Ref.</u>
Brodie and Axelrod	1.5	126
	2.4	
Carlo et al.	2.3	234
Gwilt et al.	2.7	336
Prescott et al.	2.03	347
	(mean of 8 subjects)	
Heald and Evans	2.94	322
	(mean of 10 subjects)	
Prescott et al.	2.0 $\pm$ 0.1	317
	(17 subjects)	
McGilveray et al.	3.02 $\pm$ 0.3	339
Careddu et al.	2.23 $\pm$ 0.5	315

The elimination rate constants for free (unchanged) acetaminophen and its conjugated metabolites for man have been determined by several authors<sup>265,312,319,339,348,454</sup>.

## 8.2 Protein Binding

The binding of acetaminophen to nylon<sup>72</sup> cellulose triacetate<sup>72</sup> and to dextran gels<sup>89</sup> has been described in Section 2.56. Hansch and Helmer<sup>88</sup> related this work to the octanol-water partition coefficient and ultimately to the binding of acetaminophen to natural polymers such as proteins.

Dearden and Tomlinson<sup>64</sup> have examined the binding of acetaminophen to bovine serum albumin (BSA) using a dynamic dialysis method finding the association constant to be sufficiently low to give a fairly high free drug concentration in the bloodstream over a relatively long time period.

Koss et al.<sup>285</sup> measured the binding of radiolabelled acetaminophen onto serum protein using a Sephadex filtration technique and found that about 18% of the acetaminophen is bound to the serum albumin over a wide acetaminophen concentration range. Hartshorn<sup>349</sup> quotes the amount of acetaminophen bound to the plasma proteins as about 25%.

## 8.3 Interactions with Other Drug Substances

The analgesic activity of acetaminophen has been claimed to be enhanced by the co-administration of a number of other analgesics and pharmacologically active drug substances<sup>2,285,300,321,322,350-356</sup>.

Levy and Yamada<sup>300</sup> examined the effects of salicylamide on the pharmacokinetics of acetaminophen, showing that salicylamide retards the excretion rate of acetaminophen conjugates. This was shown to be accompanied by a competitive inhibition of the formation of acetaminophen and salicylamide conjugates in the blood implying increased therapeutic availability of free acetaminophen.

Niwa and Nakayama<sup>321</sup> found that acetaminophen and antipyrine (phenazone) mutually inhibit the metabolism of each other in the rat and rabbit and showed that penetration of acetaminophen and antipyrine through excised intestine is mutually inhibited by the other drug substance.

Heald and Evans<sup>322</sup> determined the effect of antipyrine (as acetaminophen-antipyrine complex) on the plasma level of free acetaminophen in man (10 subjects). Their results suggest that antipyrine prolongs the peak plasma level of free acetaminophen.

Acetaminophen has been reported to be antagonistic to a number of drugs<sup>320,349,350,353,355,361</sup>. It has also been reported to show synergism of anti-inflammatory activity with other anti-inflammatory drugs<sup>357 to 360 and 362 to 367</sup>.

#### 8.4 Biopharmaceutics

Assessment of the bioavailability of acetaminophen has been made using both in vitro measurement of dissolution rate and in vivo pharmacokinetic methods.

Goldberg, Gibaldi and Kanig<sup>66</sup> used dissolution rate measurement to evaluate the potential increase in the bioavailability of acetaminophen after fusing it with urea to form eutectic mixtures. Lach and Cohen<sup>100</sup> carried out similar studies employing alpha and beta cyclodextrins to increase the dissolution rate of acetaminophen (see also Section 3).

Many authors have used the measurement of acetaminophen plasma levels and/or urinary levels to demonstrate its bioavailability. Mattok and co-workers<sup>75,339,345</sup> have used both in vivo and in vitro procedures and attempted to correlate the results. Levy<sup>133</sup> used the areas under the acetaminophen plasma concentration vs. time curves, to estimate the comparative systemic



availabilities of acetaminophen when administered orally as such and when administered as acetophenetidin (phenacetin).

Mattok and co-workers<sup>75,339,345</sup> used these techniques to compare the bioavailability of acetaminophen in eight commercial tablet formulations, a formulated elixir and a simple laboratory prepared solution and showed no significant differences between them. Gwilt et al.<sup>336</sup> examined the plasma levels given by seven acetaminophen formulations and later also examined an eighth formulation containing acetaminophen and sorbitol. The plasma levels of free acetaminophen given by this acetaminophen-sorbitol formulation were significantly higher than those given by the seven other formulations.

Sorbitol was claimed<sup>336</sup> to potentiate the absorption of acetaminophen and thus is claimed to enhance the antipyretic and analgesic effects of the drug<sup>96,97,98</sup>.

Walters<sup>385</sup> has critically examined these claims using in vitro methods and concludes that sorbitol does not form an absorbable complex with acetaminophen and that the enhanced activity of acetaminophen in tablets containing sorbitol may result solely from the improved dissolution rate.

Bloor and Morrison<sup>455</sup> examined the effects of solubilization of acetaminophen by Tween<sup>40</sup> (a polyoxyethylene sorbitan monopalmitate) on its rate of diffusion.

Carlo et al.<sup>234</sup> examined the effect on bioavailability obtained by formulating acetaminophen in an effervescent tablet form. The effervescent formulation gave higher and more rapidly attained plasma concentrations of acetaminophen than an ordinary non-effervescent formulation but did not maintain plasma levels as efficiently as the latter. Bru<sup>452</sup> makes similar

claims.

The effect of vehicle composition on the rectal absorption of acetaminophen from suppository formulations has been examined<sup>68,298,453</sup>.

Incorporation of enzymes having hyaluronidase and chondrosulphatase activity into acetaminophen formulations has been claimed to enhance acetaminophen absorption from both orally and rectally administered dosage forms<sup>369,370,371,372,373,374</sup>. These claims were later refuted in a study by Brustier et al.<sup>375</sup>.

The administration of acetaminophen by percutaneous absorption from solution in dialkyl sulphoxides has been reported<sup>376</sup>. Modification of drug availability can be effected by formulation as a sustained release (timed-release) dosage form. Several such formulations have been described for acetaminophen<sup>377,378,379,380,381,382</sup>. Timed release may also be effected by formulation of the drug in a microencapsulated form. This method of presentation has also been used to mask the taste of acetaminophen<sup>383,384</sup>.

## 9. Toxicity

The acute and chronic oral toxicity of acetaminophen in man and animals has been well reported<sup>3,8,67,386 to 398</sup>. Overdosing of acetaminophen can cause hepatic necrosis<sup>317,386,394,396, to 405</sup> and also in a few cases of heavy overdosing, renal insufficiency<sup>318,347,386,396,406 to 414,451</sup>. Other toxic effects have been discussed<sup>91,124,304,388,396,397,415 to 429</sup>.

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This profile attempts to cover the published literature on acetaminophen up to Chemical Abstracts, Volume 77, Issue 21

***dl*—ALPHA—TOCOPHERYL ACETATE**

*Bruce C. Rudy and Bernard Z. Senkowski*



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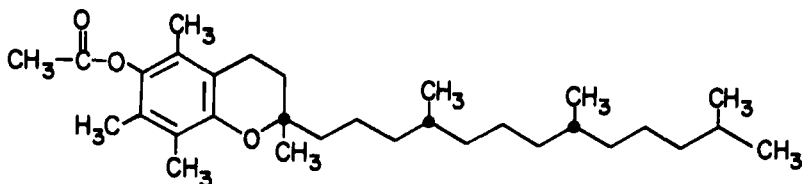
Analytical Profile - dl-Alpha-Tocopheryl Acetate

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## 1. Description

### 1.1 Name, Formula, Molecular Weight

dl-Alpha-tocopheryl acetate is a racemic mixture of 2,5,7,8-tetramethyl-2-(4',8',12'-trimethyltridecyl)-6-chromanol acetate.



$C_{31}H_{52}O_3$

Molecular Weight: 472.76

### 1.2 Appearance, Color, Odor

dl-Alpha-tocopheryl acetate occurs as a yellow, nearly odorless, clear, viscous oil.

### 1.3 Isomeric Forms

There are four possible enantiomeric pairs of diastereoisomers which result from the three asymmetric centers present in the alpha-tocopheryl acetate molecule (the asymmetric centers are marked with a small circle in the above structural formula). dl-Alpha-tocopheryl acetate contains an equimolar mixture of the eight isomers.

## 2. Physical Properties

### 2.1 Infrared Spectrum

The infrared spectrum of dl-alpha-tocopheryl acetate is presented in Figure 1 (1). The spectrum was measured with a Perkin-Elmer 621 Spectrophotometer on a capillary layer of the liquid between KBr discs. The assignments for the characteristic bands in the infrared spectrum are listed in Table I (1).

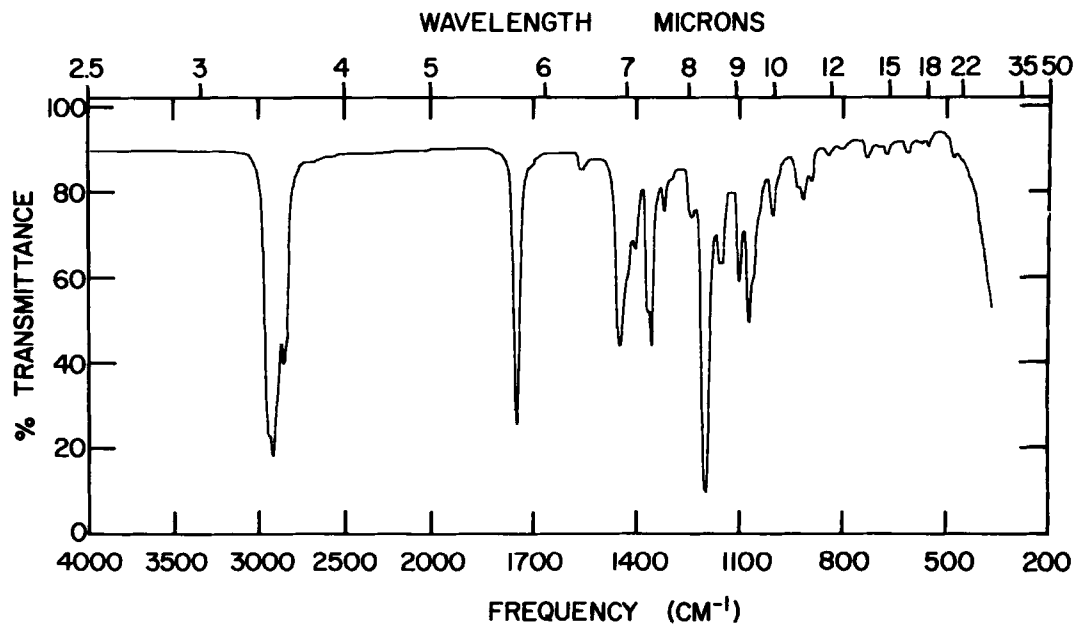
Table I

Infrared Assignments for dl-Alpha-Tocopheryl Acetate

<u>Frequency (cm<sup>-1</sup>)</u>	<u>Characteristic of</u>
2943 and 2861	CH <sub>3</sub> stretching vibrations

Figure 1

Infrared Spectrum of dl-Alpha-Tocopheryl Acetate



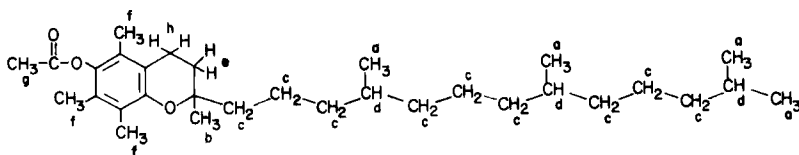
2920 and 2850	CH <sub>2</sub> stretching vibrations
1752	C=O stretching vibrations
1456	CH <sub>2</sub> and CH <sub>3</sub> deformations
1363	CH <sub>3</sub> symmetric deformations
1206	C-O-C stretching vibrations

## 2.2 Nuclear Magnetic Resonance Spectrum (NMR)

The spectrum shown in Figure 2 was obtained on a Jeolco 60 MHz NMR by dissolving 92.4 mg of dl- $\alpha$ -tocopheryl acetate in 0.5 ml of CDCl<sub>3</sub> containing tetramethylsilane as an internal reference (2). The spectral assignments are given in Table II (2).

Table II

NMR Assignments for dl- $\alpha$ -Tocopheryl Acetate



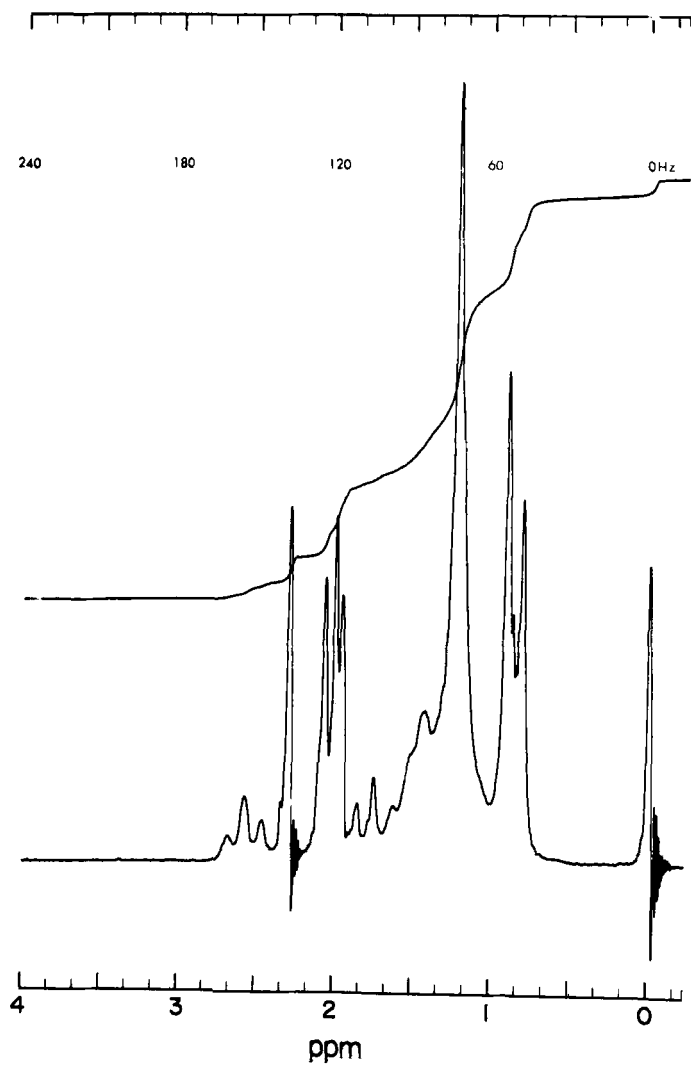
Proton	Number of Protons	Chemical Shift (ppm)	Multiplicity
a	12	0.85	Doublet ( $J_{a-d} = 5$ Hz)
b	3	1.21	Singlet
c&d	21	1.0-1.6	Complex Multiplet
e	2	1.75	Triplet ( $J_{e-h} = 6.8$ Hz)
f	9	1.96, 2.00, 2.07	Three Overlapping singlets
g	3	2.29	Singlet
h	2	2.57	Triplet ( $J_{h-e} = 6.8$ Hz)

## 2.3 Ultraviolet Spectrum (UV)

When the UV spectrum of dl- $\alpha$ -tocopheryl acetate was scanned from 350 to 220 nm, two maxima and two minima were observed. One maximum is located at 285 nm ( $\epsilon = 2.24 \times 10^3$ ) with a shoulder at 287 nm ( $\epsilon = 2.21 \times 10^3$ ) and the other maximum occurs at 279 nm ( $\epsilon = 1.98 \times 10^3$ ). The minima are located at 281 nm and 253 nm. The spectrum shown in Figure 3 was obtained from a solution of 12.411 mg of dl- $\alpha$ -tocopheryl acetate per 100 ml of cyclohexane(3).

Figure 2

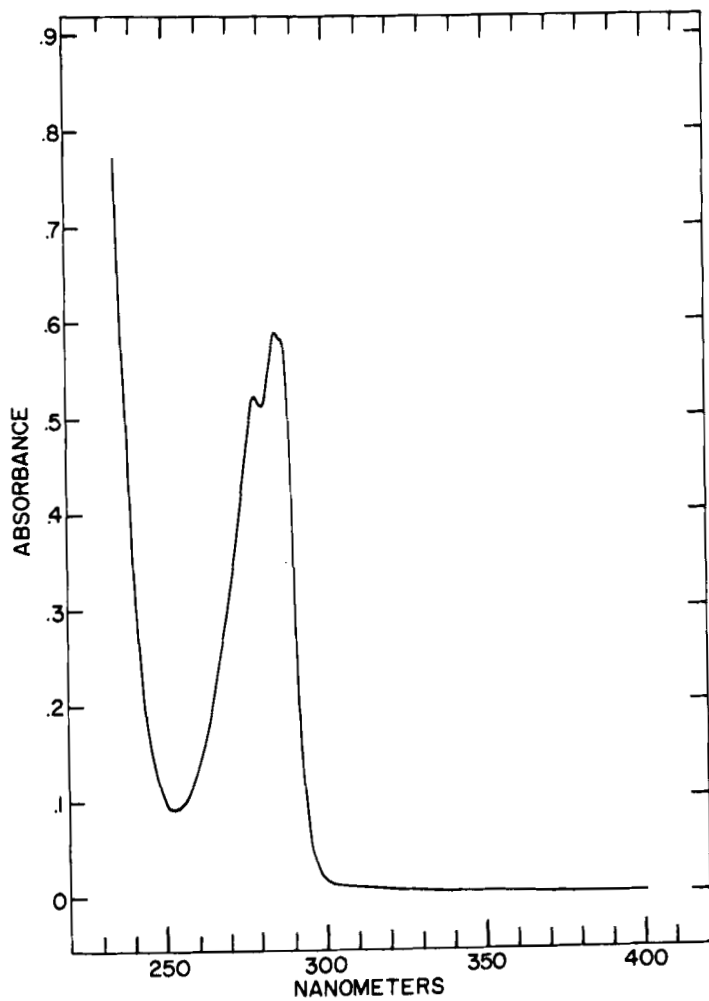
NMR Spectrum of dl-Alpha-Tocopheryl Acetate



dl- $\alpha$ -TOCOPHERYL ACETATE

Figure 3

Ultraviolet Spectrum of dl- $\alpha$ -Tocopheryl Acetate



#### 2.4 Mass Spectrum

The mass spectrum of dl-alpha-tocopheryl acetate was obtained using a CEC 21-110 mass spectrometer with an ionizing energy of 70 eV. The output from the mass spectrometer was analyzed and presented in the form of a bar graph, shown in Figure 4, by a Varian 100 MS dedicated computer system (4). The mass spectrum of dl-alpha-tocopheryl acetate is characterized by the absence of numerous fragmentation processes. The molecular ion peak occurs at m/e 472. The base peak at m/e 430 occurs from the loss of ketene from the molecular ion. Fragmentation of the non-aromatic ring occurs, yielding a peak at m/e 207 (with the acetate group present) and a peak at m/e 165 (after loss of the ketene group). An in-depth analysis of the mass spectra of tocopherols has recently been published by Scheppele et al. (5).

#### 2.5 Optical Rotation

dl-Alpha-tocopheryl acetate is an equimolar mixture of the optical isomers of alpha-tocopheryl acetate and therefore exhibits no optical rotation.

#### 2.6 Melting Range

dl-Alpha-tocopheryl acetate is an oil at room temperature. It solidifies at a temperature of -27.5°C (6).

#### 2.7 Differential Scanning Calorimetry (DSC)

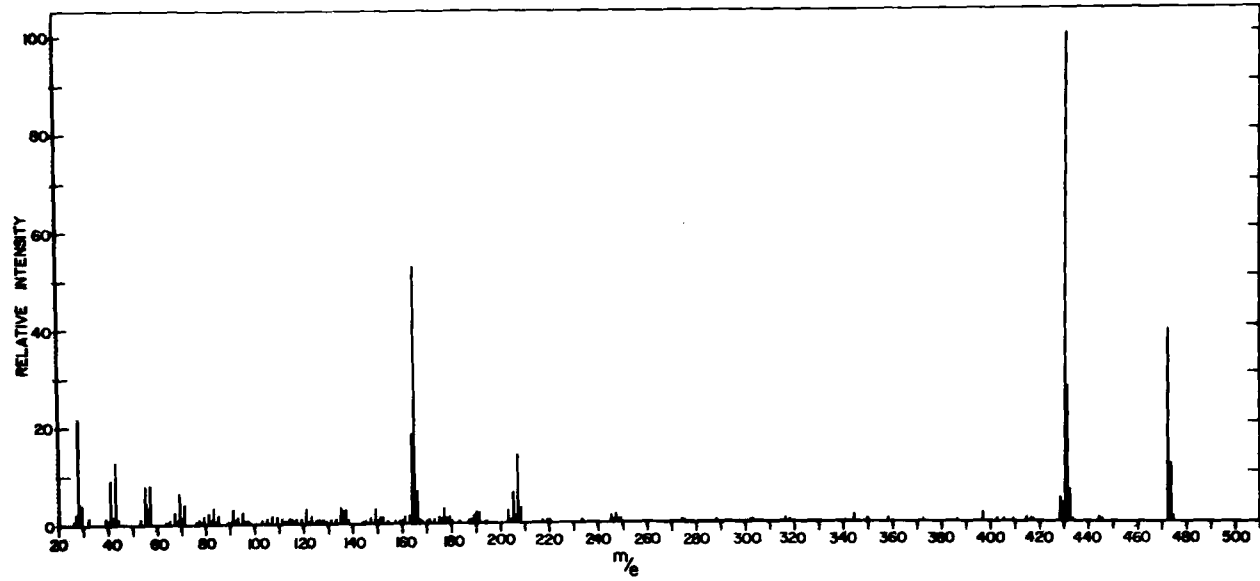
A DSC scan was run by cooling the head of the DSC which held the sample pan containing the dl-alpha-tocopheryl acetate to -90°C. After holding the temperature at -90°C for 30 minutes, the temperature was increased at a rate of 10°/minute. Only an extremely weak endothermic transition was observed starting at about -44°C. It appears that when dl-alpha-tocopheryl acetate solidifies it forms a glass instead of a crystalline material (7).

#### 2.8 Thermogravimetric Analysis (TGA)

The TGA of dl-alpha-tocopheryl acetate in a nitrogen atmosphere showed no weight loss from ambient to 210°C. A single weight loss corresponding to 100% of the sample occurred between 210° and 370°C (7).

Figure 4

Mass Spectrum of dl-Alpha-Tocopheryl Acetate





### 3. Synthesis

dl-Alpha-tocopheryl acetate is prepared by the reaction scheme shown in Figure 5. Trimethylhydroquinone is condensed with racemic isophytol to yield dl-alpha-tocopherol which is then acetylated (8).

### 4. Stability Degradation

dl-Alpha-tocopheryl acetate is practically unaffected by the oxidizing influence of air and ultraviolet light (6). When it is refluxed in acidic and basic solutions in the absence of oxygen, the molecule is hydrolyzed to the free dl-alpha-tocopherol. If oxygen is present, the dl-alpha-tocopherol, once formed, will oxidize rapidly to the quinone. The rate of oxidation is much faster in the basic solution.

### 5. Drug Metabolic Products

dl-Alpha-tocopheryl acetate is metabolized as outlined in Figure 6 (9,10). The ester is readily converted in the animal to free alpha-tocopherol which is then further metabolized to alpha-tocopherol quinone and an alpha-tocopherol dimer. The alpha-tocopherol quinone may be reduced to the corresponding hydroquinone or further oxidized to alpha tocopheronic acid (9,10,11).

### 6. Methods of Analysis

#### 6.1 Elemental Analysis

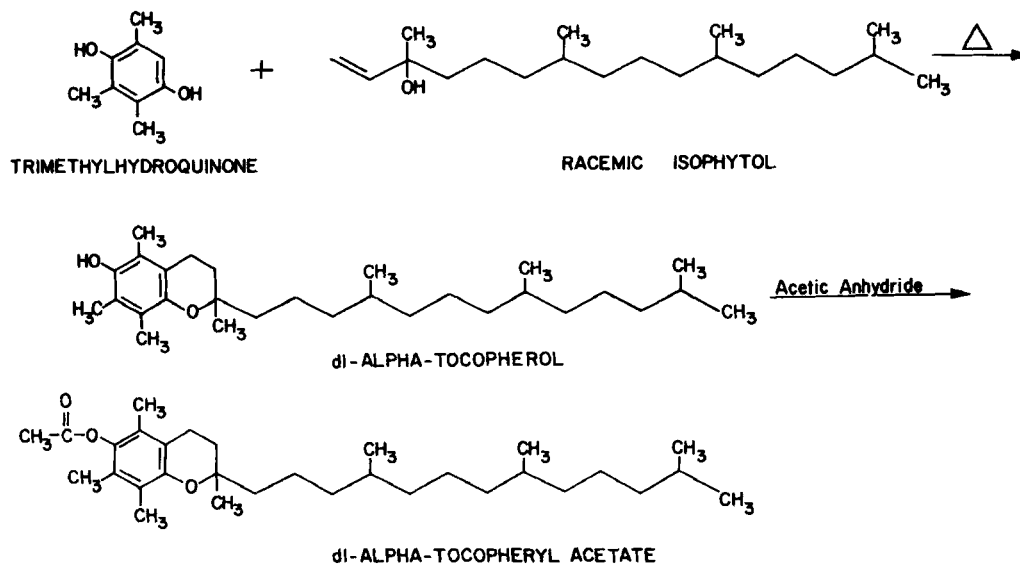
The results from the elemental analysis of dl-alpha-tocopheryl acetate are listed below (12).

<u>Element</u>	<u>% Theory</u>	<u>% Found</u>
C	78.76	78.55
H	11.09	11.05

#### 6.2 Thin Layer Chromatographic Analysis (TLC)

A TLC system which can be used to separate dl-alpha-tocopheryl acetate from its major metabolites is as follows. The sample is applied to a Silica Gel G plate and subjected to ascending chromatography using cyclohexane:diethyl ether (4:1) as the developing solvent. After the solvent has ascended 10 to 15 cm, the plate is air dried, sprayed with concentrated sulfuric acid, and warmed in an oven at 105°C for 5 minutes. The approximate R<sub>f</sub> values

Figure 5  
Synthesis of dl-Alpha-Tocopheryl Acetate



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are listed below (13).

alpha tocopherol	0.5
alpha tocopheryl acetate	0.7
alpha tocopherol quinone	0.9

### 6.3 Gas-Liquid Chromatographic Analysis (GLC)

A recent collaborative study has shown dl-alpha-tocopheryl acetate may readily be separated and assayed by GLC (14). The pertinent experimental conditions as well as the retention times are given in Table III.

Table III

#### GLC Method for dl-Alpha-Tocopheryl Acetate

Column:	4-8 feet, 2-3 mm i.d., Pyrex or stainless steel
Support:	Silanized Diatomaceous Earth
Stationary Phase:	5-10% SE-30
Detector:	Hydrogen flame ionization
Temperature (°C)	
Injection Port:	300
Column:	280
Detector:	300
Carrier Gas Flow Rate:	~40 ml/min. of nitrogen
Quantities Injected:	~10 mcg in n-hexane
Retention Time (minutes)	
alpha-tocopherol	22
alpha-tocopheryl acid succinate	23
alpha-tocopheryl acetate	26
dotriacontane (internal standard)	30

### 6.4 Direct Spectrophotometric Analysis

Direct spectrophotometry may be carried out on dl-alpha-tocopheryl acetate provided there are no interferences present. The maxima for dl-alpha-tocopheryl acetate are dependent on the choice of solvent used. If cyclohexane is used as the solvent, the maximum at 285 nm may be used for quantitation.

### 6.5 Colorimetric Analysis

An indirect method for the analysis of dl-alpha-tocopheryl acetate utilizes the Emmerie-Engle colorimetric procedure (15). The dl-alpha-tocopheryl acetate is base hydrolyzed in anhydrous ethanol to the free tocopherol. The solution is acidified to prevent air oxidation, water is added, and the dl-alpha-tocopherol is extracted into diethyl ether. The ether is evaporated under nitrogen and the residue is immediately dissolved in anhydrous ethanol. Ferric chloride is added along with 2,2'-bipyridine, both in anhydrous ethanol. The mixture is shaken vigorously and timed for 10 minutes. The absorbance of the red solution is measured at 520 nm (16,17). A review of the Emmerie-Engle and ferric sulfate titrimetric methods for Vitamin E was published by Lehman (18).

### 6.6 Spectrofluorometric Analysis

The intense ultraviolet fluorescence exhibited by alpha-tocopherol has provided the basis for a simple and extremely sensitive method for the determination of free alpha-tocopherol and alpha-tocopheryl acetate in plasma (19,20). Two ml of plasma are diluted with 2 ml of water and 4 ml of ethanol and then extracted with 8 ml of hexane. The free alpha-tocopherol is determined by diluting a 1 ml aliquot of the hexane phase with 3 ml of ethanol and measuring the intensity of the fluorescence produced at 340 nm by exciting the sample at 295 nm. The alpha tocopheryl acetate is determined by difference after hydrolyzing a 4 ml aliquot of the hexane phase with  $\text{LiAlH}_4$  to convert any acetate to alpha-tocopherol and measuring the intensity of the fluorescence as above. A plot of fluorescence versus concentration of alpha tocopherol was linear over the range of 0.6  $\mu\text{g/ml}$  through 40  $\mu\text{g/ml}$  (20). The limit of detection is about 0.01  $\mu\text{g/ml}$  (19).

### 6.7 Titrimetric Analysis

dl-Alpha-tocopheryl acetate (about 250 mg) is dissolved in 25 ml of anhydrous ethanol, 20 ml of 5N ethanolic sulfuric acid is added and the solution refluxed for 3 hours to effect complete hydrolysis to the free dl-alpha-tocopherol. After the solution is cooled, it is transferred to a 200-ml volumetric flask and diluted to volume with 50 ml of 0.5N ethanolic sulfuric acid and 20 ml of water. Two drops of diphenylamine T.S. are added and the

solution is titrated with 0.01N ceric sulfate until a blue end point is reached which persists for 10 seconds. A blank is run and any necessary volume correction made. Each ml of 0.01N ceric sulfate is equivalent to 2.3638 mg of dl-alpha-tocopheryl acetate (17).

7. Acknowledgments

The authors wish to acknowledge Mrs. A. M. Ormsby for typing many of the Analytical Profiles and Mrs. L. B. Rubia for drawing and lettering many of the figures. The help afforded by the Scientific Literature Department and the Research Records Office of Hoffmann-La Roche Inc. in the literature searches also is gratefully acknowledged.

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**AMITRIPTYLINE HYDROCHLORIDE**

*Kenneth W. Blessel, Bruce C. Rudy, and Bernard Z. Senkowski*



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Analytical Profile - Amitriptyline Hydrochloride

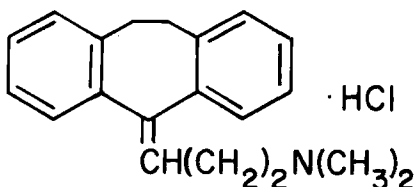
1. Description
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# AMITRIPTYLINE HYDROCHLORIDE

## 1. Description

### 1.1 Name, Formula, Molecular Weight

Amitriptyline hydrochloride is 10,11-dihydro-N,N-dimethyl-5H-dibenzo[a,d]cycloheptene- $\Delta^5$ ,  $\gamma$ -propylamine hydrochloride.



$\text{C}_{20}\text{H}_{23}\text{N} \cdot \text{HCl}$

Molecular Weight: 313.87

### 1.2 Appearance, Color, Odor

Amitriptyline hydrochloride is an odorless, off-white crystalline powder.

## 2. Physical Properties

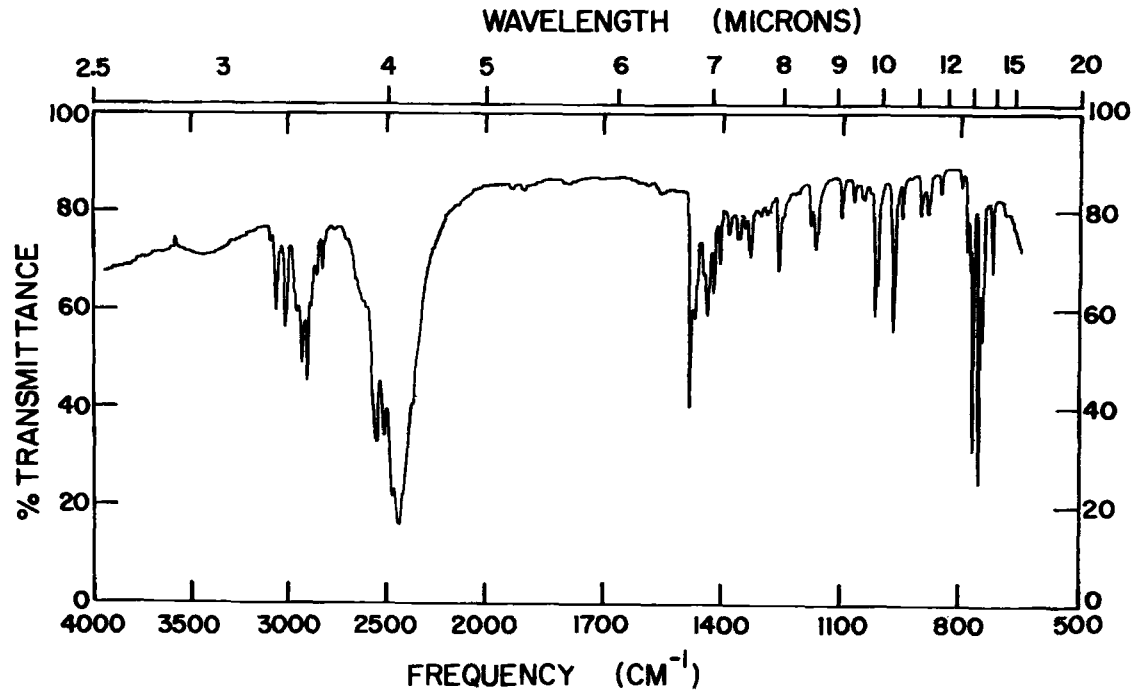
### 2.1 Infrared Spectrum

The infrared spectrum of amitriptyline hydrochloride is shown in Figure 1 (1). The sample was dispersed in fluorolube for the region of 4000-1350  $\text{cm}^{-1}$  and in mineral oil to record the spectrum in the region of 1350-600  $\text{cm}^{-1}$ . The following assignments of bands in the spectrum have been made (1).

<u>Band</u>	<u>Assignment</u>
3057 $\text{cm}^{-1}$	Aromatic CH stretch
2949 and 2825 $\text{cm}^{-1}$	Asymmetric and symmetric $\text{CH}_3$ stretch
2921 and 2852 $\text{cm}^{-1}$	Asymmetric and symmetric $\text{CH}_2$ stretch
2545-2428 $\text{cm}^{-1}$	Characteristic of HCl salt of tertiary amine
767 and 757 $\text{cm}^{-1}$	Four adjacent hydrogens on benzene ring

Figure 1

Infrared Spectrum of Amitriptyline Hydrochloride



## AMITRIPTYLINE HYDROCHLORIDE

### 2.2 Nuclear Magnetic Resonance Spectrum (NMR)

The NMR spectrum of amitriptyline hydrochloride is shown in Figure 2 (2). The spectrum was recorded using a solution of 54.7 mg/0.5 ml in  $\text{CDCl}_3$ . The spectral assignments are shown in Table I (2).

Table I

#### NMR Spectral Assignments for Amitriptyline Hydrochloride

<u>Proton Identification</u>	<u>Total No. of Each</u>	<u>Chemical Shift (ppm)</u>	<u>Multiplicity</u>
N,N-dimethyl	6	2.65	Singlet
Protons on 7-membered ring and methylene protons	8	2.75-3.34	Multiplet (unresolved)
Methine protons	1	5.8	Triplet
Aromatic protons	8	7.2	Multiplet
Proton of Hydrochloride	1	12.5	Singlet (broad)

### 2.3 Ultraviolet Spectrum

The ultraviolet spectrum of amitriptyline hydrochloride is shown in Figure 3 (3). The spectrum was recorded on a solution which contained 1.00 mg in 100 ml of 0.1N HCl. A maximum was observed at about 239 nm ( $\epsilon = 1.4 \times 10^4$ ) and a minimum at 228-231 nm.

### 2.4 Fluorescence Spectrum

The excitation and emission spectra of amitriptyline hydrochloride are shown in Figure 4 (4). The sample was dissolved in methanol at a concentration of 1 mg/ml and the spectra were recorded using a Farrand MK-1 recording spectrofluorometer. Excitation at 302 nm produced emission with a maximum at 357 nm.

### 2.5 Mass Spectrum

The low resolution mass spectrum of amitriptyline is shown in Figure 5 (5). The spectrum was recorded on a CEC 21-110 mass spectrometer using an ionizing energy of 70 eV, which was interfaced with a Varian data system

Figure 2

NMR Spectrum of Amitriptyline Hydrochloride

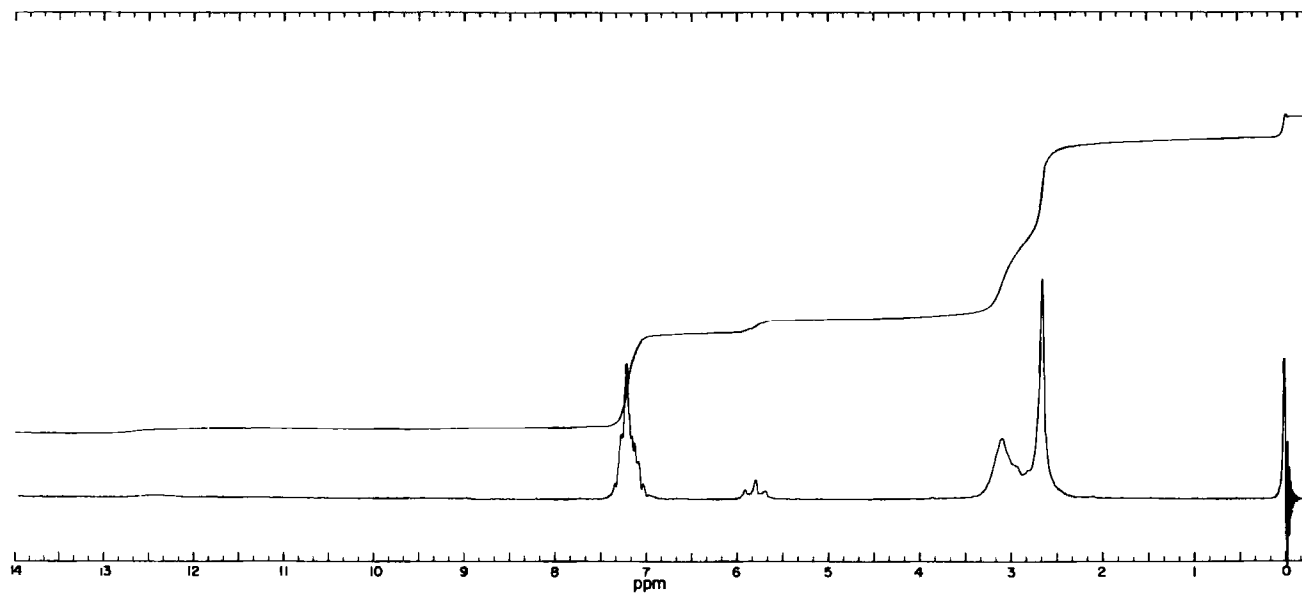


Figure 3

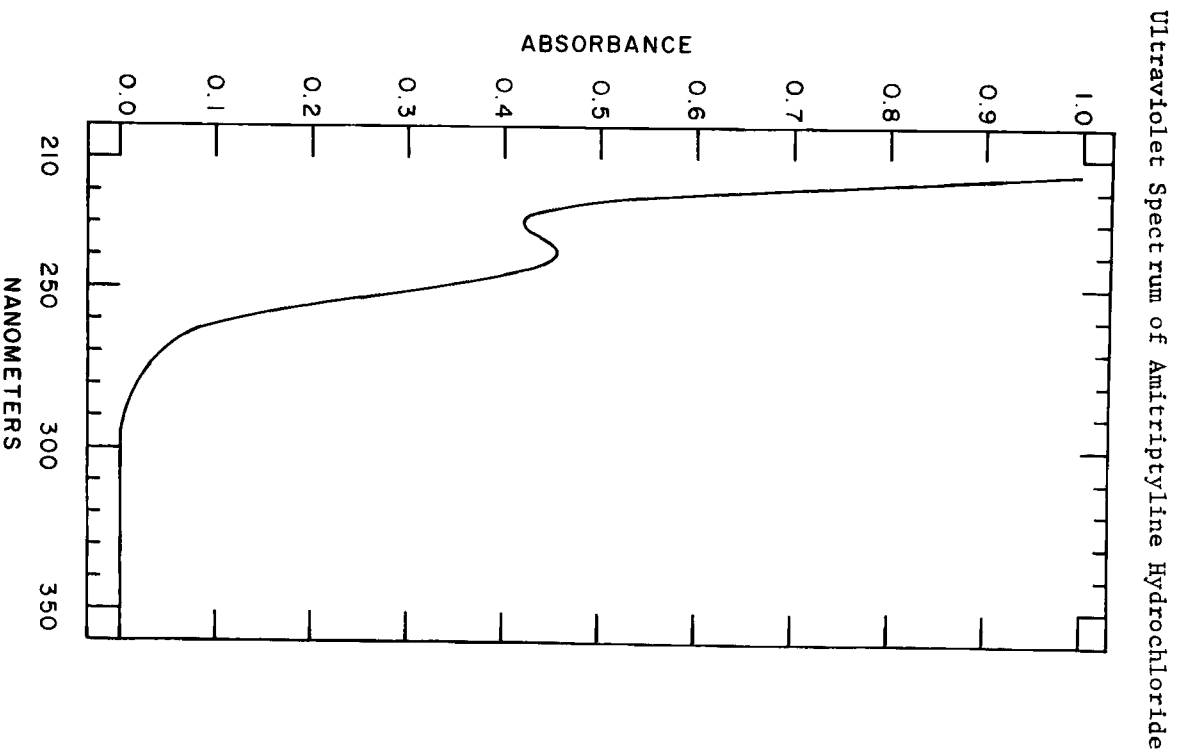


Figure 4

Fluorescence Spectra of Amitriptyline Hydrochloride

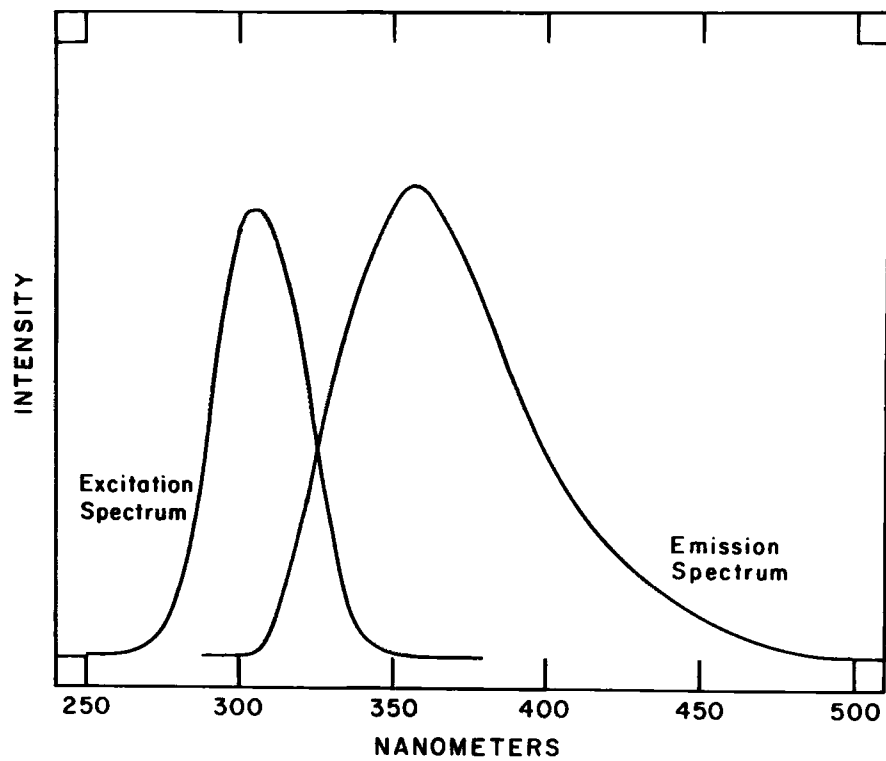
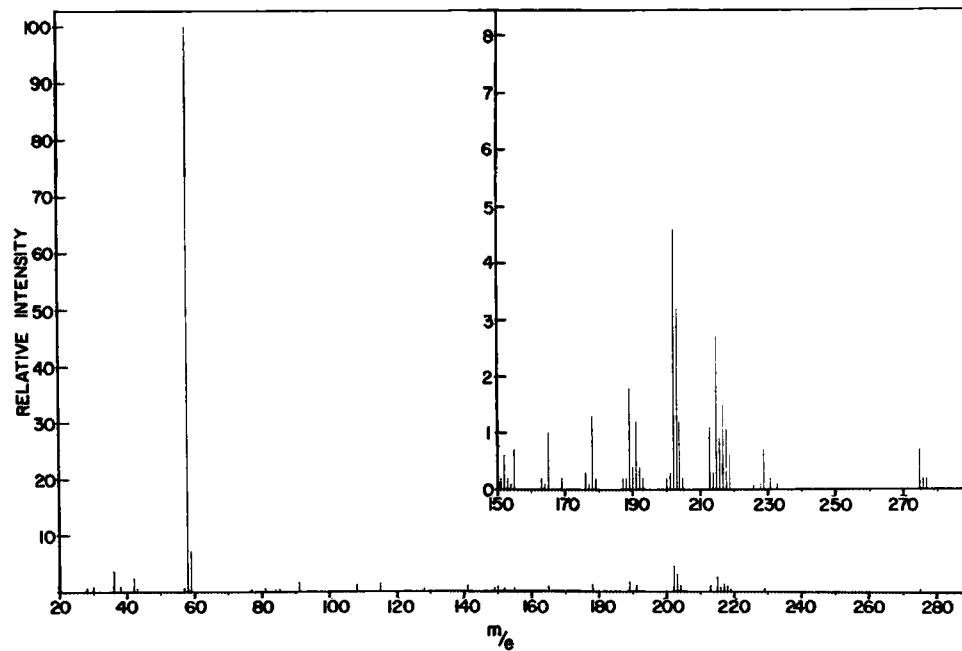


Figure 5

## Mass Spectrum of Amitriptyline





100 MS. The data system accepted the output of the spectrometer, calculated the masses, compared their intensities to the base peak and plotted this data as a series of lines whose heights were proportional to the intensities.

The molecular ion was measured at  $m/e$  277 (free base). The characteristic feature of this compound is its strong tendency to lose H in order to attain aromaticity and/or ring closure. The main fragments are the loss of  $N(CH_3)_2$  and  $CH_2N(CH_3)_2$  from the side chain to form  $m/e$  233 and 219 respectively. Each of these species then loses hydrogens, giving rise to  $m/e$  231, 217, and 215. Complete loss of the side chain gives rise to  $m/e$  192 and  $m/e$  85. The base peak occurs at  $m/e$  58 and is due to  $CH_2N(CH_3)_2$  (5). A high resolution spectrum was found to be fully compatible with the low resolution scan.

## 2.6 Optical Rotation

Amitriptyline hydrochloride does not exhibit optical activity.

## 2.7 Melting Range

The melting range reported in USP XVIII is 195-198°C when a Class I procedure is used (6).

## 2.8 Differential Scanning Calorimetry (DSC)

The DSC curve for amitriptyline hydrochloride at a scan rate of 10°C/min. is shown in Figure 6 (7). The curve was recorded with a Perkin Elmer DSC-1B under an atmosphere of nitrogen. A single endotherm was observed, the extrapolated onset of melting occurring at  $193.0 \pm 0.2^\circ\text{C}$  and the peak at  $197.5 \pm 0.2^\circ\text{C}$ . All temperatures are corrected. The value calculated for  $\Delta H_f$  was 6.7 kcal/mole for the melting endotherm.

## 2.9 Thermogravimetric Analysis (TGA)

The TGA curve for reference standard amitriptyline hydrochloride exhibited no loss of weight from 30-195°C. A single weight loss occurred in the temperature range of 195-317°C which accounted for 100% of the sample weight (7).

## 2.10 Solubility

The solubility data obtained for reference standard amitriptyline hydrochloride is listed in Table II (8).

AMITRIPTYLINE HYDROCHLORIDE

Figure 6

DSC Curve of Amitriptyline Hydrochloride

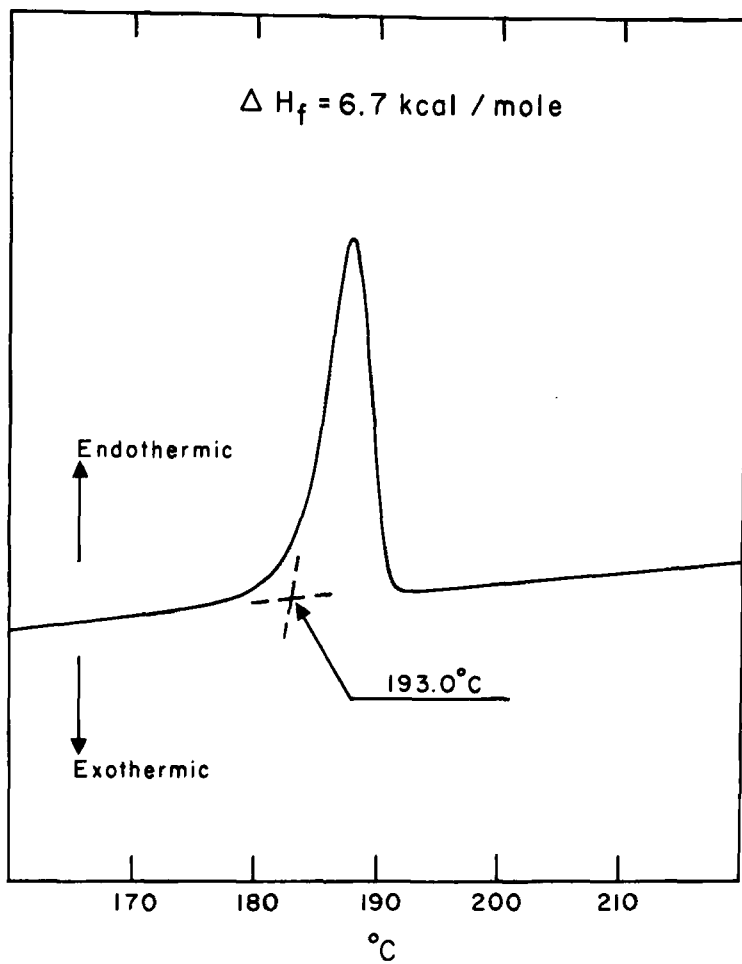


Table II

## Solubility of Amitriptyline Hydrochloride

<u>Solvent</u>	<u>Solubility (mg/ml)</u>
petroleum ether (30-60°C)	0.30
diethyl ether	0.50
water	>500.
2-propanol	53.
3A alcohol	313.
chloroform	>500.
95% ethanol	>500.
benzene	5.0
methanol	>500

2.11 Crystal Properties

The x-ray powder diffraction data for a sample of reference standard amitriptyline hydrochloride is given in Table III (9). The operating parameters of the instrument are given below.

Instrumental Conditions

## General Electric Model XRD-6 Spectrogoniometer

Generator:	50 KV, 12-1/2 MA
Tube target:	Copper
Radiation:	Cu $K\alpha$ = 1.542 Å
Optics:	0.1° Detector slit
	M.R. Soller slit
	3° Beam slit
	0.0007" Ni filter
	4° take off angle
Goniometer:	Scan at 0.2° 2θ per minute
Detector:	Amplifier gain - 16 course,
	8.7 fine
	Sealed proportional counter
	tube and DC voltage at
	plateau
	Pulse height selection $E_L$ -
	5 volts
	Eu - out
	Rate meter T.C. 4
	2000 C/S full scale

# AMITRIPTYLINE HYDROCHLORIDE

Recorder: Chart speed 1 inch per 5 minutes  
 Samples: Prepared by grinding at room temperature

Table III

Interplanar Spacings in Amitriptyline Hydrochloride  
 from Powder Diffraction Data

<u>2θ</u>	<u>d(Å)*</u>	<u>I/I<sub>0</sub>**</u>	<u>2θ</u>	<u>d(Å)*</u>	<u>I/I<sub>0</sub>**</u>
11.50	7.69	21	29.36	3.04	3
12.78	6.93	10	29.52	3.03	2
13.46	6.58	4	30.10	2.97	4
14.86	5.96	16	30.96	2.89	4
15.66	5.66	9	31.28	2.86	7
16.06	5.52	4	31.74	2.82	6
16.34	5.42	15	31.98	2.80	3
16.72	5.30	18	32.46	2.76	5
17.72	5.01	1	32.76	2.73	7
18.58	4.78	74	33.80	2.65	6
18.90	4.70	13	34.12	2.63	3
19.26	4.61	36	34.98	2.57	2
20.24	4.39	9	35.30	2.54	3
20.94	4.24	100	36.00	2.49	3
21.34	4.16	10	38.26	2.35	3
21.87	4.06	4	39.02	2.31	6
22.94	3.88	60	39.66	2.27	2
23.42	3.80	7	40.04	2.25	1
24.32	3.66	9	40.54	2.23	1
24.88	3.58	3	41.22	2.19	2
25.78	3.46	19	41.88	2.16	1
26.62	3.35	16	42.06	2.15	2
27.30	3.27	9	43.24	2.09	3
27.58	3.23	3	43.88	2.06	2
28.16	3.17	9	44.22	2.05	2
29.00	3.08	4	44.70	2.03	2
			45.30	2.00	2

$$*d = (\text{interplanar distance}) \frac{n\lambda}{2 \sin \theta}$$

\*\*I/I<sub>0</sub> = relative intensity (based on highest intensity of 100)

### 2.12 Dissociation Constant

The dissociation constant for amitriptyline hydrochloride was determined using a graphical method involving the pH dependence of the water solubility. The value for the pKa determined by this method was 9.4 (10).

### 3. Synthesis

Two synthetic routes to amitriptyline are shown in Figure 7. The first, reaction sequence I (11,12), involves the addition of a Grignard reagent followed by dehydration with HCl or acetyl chloride, while II (13) uses a cyclopropyl Grignard reagent followed by ring opening with dimethylamine to form the desired compound. A number of alternative syntheses have been described in the literature (14-17).

### 4. Stability Degradation

The stability of amitriptyline hydrochloride, in the bulk form, was studied under conditions of elevated temperature or exposure to light (18). It was found to be stable at room temperature and at 45°C for a period in excess of two months. It showed some decomposition after two months at 100°C, and when exposed to light, as evidenced by the formation of a brownish discoloration of the powder. A 1% aqueous solution was found to be stable at 0°C, room temperature, and 45°C, as well as for a period of 60 hours at 100°C.

### 5. Drug Metabolic Products

Hucker and Porter (19) demonstrated that very little of a dose of amitriptyline is excreted unchanged in humans. Other investigators (20-23) have demonstrated the presence of the major metabolic products shown in Figure 8. Facino and Corona (20) have demonstrated metabolites I, III, IV, the two isomers of V, and VI in the organs of rabbits. Eschenoff and Rieder (21) demonstrated metabolites I, II, and V and in addition reported the existence of the N-oxide metabolite (VII) in studies on rats and humans. They reported that the metabolism of amitriptyline in these two species is nearly identical (24). In addition, Facino and Corona (25) later reported the existence of an acidic metabolite to which they ascribed the structure of the carboxylic acid which would be formed by oxidation deamination of the drug.

Figure 7

Syntheses of Amitriptyline

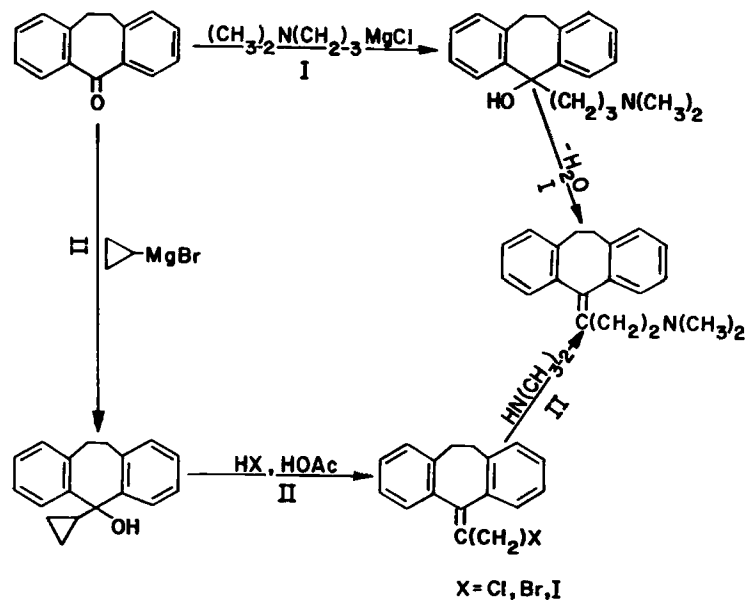
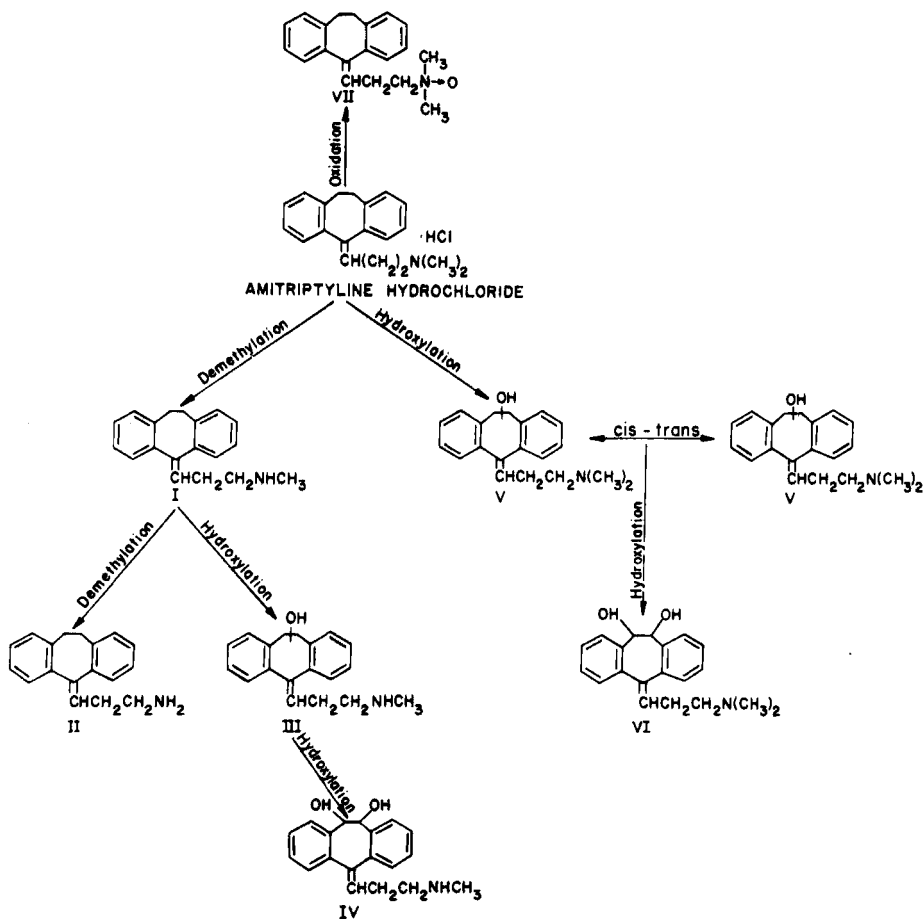


Figure 8

Metabolic Products of Amitriptyline Hydrochloride



## 6. Methods of Analysis

### 6.1 Elemental Analysis

The results of an elemental analysis of a sample of reference standard amitriptyline hydrochloride is presented in Table IV below (32).

<u>Element</u>	<u>Theory %</u>	<u>Found %</u>
C	76.53	76.44
H	7.70	7.79
N	4.47	4.50
Cl	11.30	11.26

### 6.2 Phase Solubility Analysis

A phase solubility analysis for amitriptyline hydrochloride is shown in Figure 9. The solvent used was acetone and the extrapolated solubility was 17.81 mg/g (8).

### 6.3 Thin Layer Chromatographic Analysis

A number of thin layer chromatographic systems for amitriptyline hydrochloride have been described in the literature. A representative number of these systems are shown in Table V. Methods of detection were not included since these are many times determined by the individual preferences of the analyst or the particular separation desired (26).

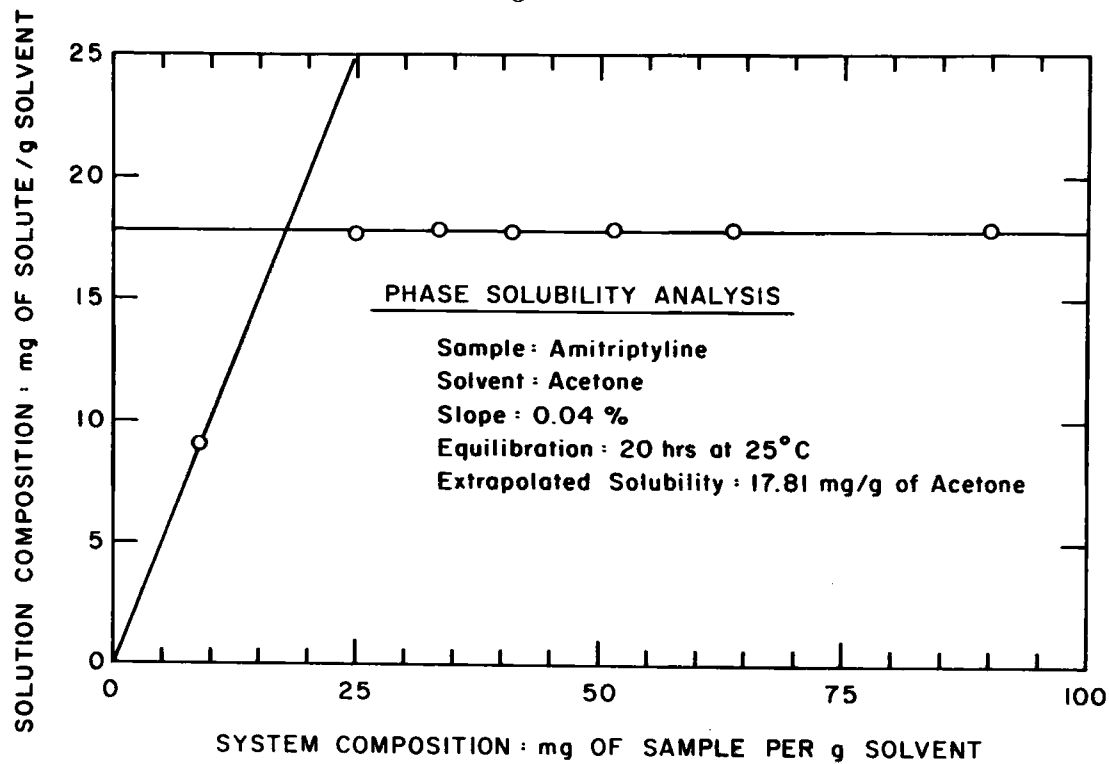
Table V

#### TLC Systems for Amitriptyline Hydrochloride

<u>Adsorbent</u>	<u>Solvent System</u>	<u>R<sub>f</sub></u>	<u>Reference</u>
Silica Gel	benzene:dioxane:NH <sub>3</sub> (60:35:5)	0.74	27
Silica Gel	ethanol:acetic acid: H <sub>2</sub> O (50:30:20)	0.65	27
Silica Gel	methanol:butanol (60:40)	0.37	27
Silica Gel + 0.1M NaOH	cyclohexane:benzene: diethylamine (75:15:10)	0.72	28



Figure 9



## AMITRIPTYLINE HYDROCHLORIDE

Silica Gel + 0.1M NaOH	methanol	0.50	28
Silica Gel + 0.1M NaOH	acetone	0.34	28
Silica Gel + 0.1M KHSO <sub>4</sub>	methanol	0.41	28
Silica Gel + 0.1M KHSO <sub>4</sub>	95% ethanol	0.28	28

### 6.4 Gas-Liquid Chromatographic Analysis (GLC)

A GLC method for the quantitative determination of amitriptyline has been described (29). The method was developed for measuring the amitriptyline content of plasma. The lower limit of detection is 20 ng/ml. The chromatographic conditions are given below.

Column -	5 ft. x 1/4 in. silanized glass
Support -	Chromosorb W (80-100 mesh)
Liquid Phase -	1% polyvinyl pyrrolidinone and 3% Versamid 900
Detection -	Flame ionization
Oven Temperature -	205°C
Detector	
Temperature -	240°C
Injection Port	
Temperature -	Maximum
Carrier Gas -	Nitrogen, 50 ml/min.

### 6.5 Colorimetric Analysis

Amitriptyline hydrochloride can be determined colorimetrically using the methyl orange reaction. The method involves buffering the solution containing the compound at a pH value of 4.3, adding the methyl orange and extracting the resulting complex into ethylene dichloride. The absorbance of the ethylene dichloride extract is measured at about 430 nm and the amount of amitriptyline calculated by comparison with a calibration curve prepared from pure amitriptyline. This method can be used to determine amitriptyline in the presence of its N-demethylated metabolites by the addition of acetic anhydride before extraction since primary and secondary amines do not react with methyl orange in the presence of acetic anhydride (30).

### 6.6 Fluorescence Analysis

A sensitive fluorimetric assay has been developed for amitriptyline hydrochloride in biological samples (31).

The biological material to be analyzed is homogenized and an equal volume of methanol added. Then 0.2 g of borax, 15 ml of heptane and 1 ml of distilled water is added to a 1 ml aliquot of the methanolic sample. The heptane layer after separation, is then extracted with perchloric acid. The acid extract is then heated in a boiling water bath for 10 min. and cooled. The fluorescence of the carbonium ion generated by the heating process is then measured at 555 nm using an activation wavelength of 305 nm. The fluorescence intensity was found to be linear with amitriptyline concentration in the range of 0.05-5.0 mcg/ml.

#### 6.7 Titrimetric Analysis

A non-aqueous titration with perchloric acid in acetic acid is the preferred method for the analysis of bulk amitriptyline hydrochloride. The sample is dissolved in glacial acetic acid. Then mercuric acetate T.S. and crystal violet T.S. are added. The solution is titrated with 0.1N perchloric acid to a green end-point. Each ml of 0.1N perchloric acid is equivalent to 31.39 mg of amitriptyline hydrochloride (6).

#### 7. Acknowledgments

The authors wish to acknowledge the assistance of the Research Records Office and the Scientific Literature Department of Hoffmann-La Roche Inc.

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# **DIGITOXIN**

*Ivan M. Jakovljevic*

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## 1. DESCRIPTION

Digitoxin is a cardiotonic glycoside obtained from *Digitalis purpurea* Linné, *Digitalis lanata* Ehrhart, and other suitable species of *Digitalis* leaves.

1.1 Registered Names

Digitoxin is designated by the following names:

CARDIGIN (Nat.Drugs), CRYSTODIGIN (Lilly), DIGICORYL (Roussel), DIGILONG (Boehringer), DIGIMERCK (Merck), DIGIPAN, DIGISIDIN (Winthrop), DIGITALINE NATIVELLE (Varick), DIGITORA (Upjohn), DIGITOXIN (Sandoz), DIGITOXOSIDE (W.H.O.), DIGITRIN (Astra), LANATOXIN (Beiersdorf), PURODIGIN (Wyeth), PURPUREN, PURPURID (Promonta).

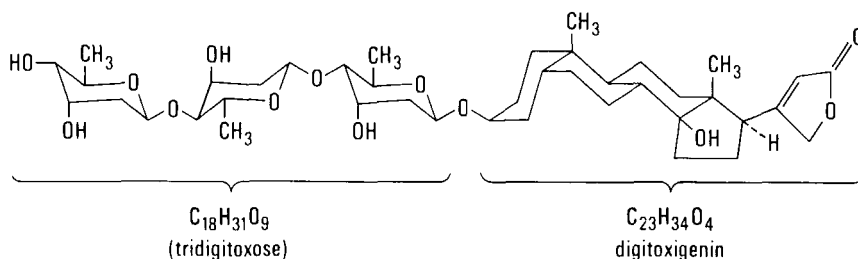
1.2 Chemical Name

3 $\beta$ -(D-Digitoxosyl-D-digitoxosyl-D-digitoxosyl-oxy)-14 $\beta$ -hydroxy-5 $\beta$ -card-20(22)-enolide.

1.3 Formula, Structure, Molecular Weight

C<sub>41</sub>H<sub>64</sub>O<sub>13</sub>

Mol.Wt. 764.94



THE CONFORMATIONAL ARRANGEMENT

Digitoxin belongs to the cardenolide series, which consists of a steroid nucleus with a 5-membered unsaturated lactone ring at C-17.

As in most other glycosides, the sugar is present in the six-membered ring form (pyranoid

form) with the chair conformation.

The steroid framework is considerably bent at either end of the molecule, which is an important steric requirement for cardiotonic activity. Saturation of the lactone ring greatly reduces the cardiotonic activity. The unsaturated lactone ring must be attached in the  $\beta$ -configuration. Epimerization reduces pharmacological activity by at least 400 times. Opening the lactone ring by alkaline hydrolysis also results in loss of activity<sup>1</sup>.

#### 1.4 Appearance

Very small elongated, rectangular plates from diluted ethanol, or microcrystalline powder, white or pale buff, odorless, very bitter taste.

### 2. PHYSICAL PROPERTIES

#### 2.1 Infrared Spectrum

The infrared (IR) spectrum of digitoxin, USP reference standard, is given in Fig.1. The IR spectrum was taken in a KBr pellet on a Beckman IR-12 spectrometer.

IR spectral assignments of digitoxin are as follows<sup>2</sup>:

<i>Wavelength of Absorption (<math>CM^{-1}</math>);</i>	<i>Vibration Mode:</i>
3575	-OH stretch (non-hydrogen bonded)
3440	-OH stretch (hydrogen bonded)
2960, 2935	CH stretch, $CH_3$ , $CH_2$ stretch
1740	C=O stretch ( $\alpha, \beta$ unsaturated lactone)
1630	CH stretch (conjugated double bond)
1448, 1403, 1379, 1367, 1348	CH deformation ( $CH_3$ , $CH_2$ , C- $CH_3$ , CH)

1162	3 <sup>0</sup> -OH deformation
1125	2 <sup>0</sup> -OH deformation
1075	C-O- stretch (glycosidic ether)
1060	C-O- stretch (cyclic ether oxygens)
1040	1 <sup>0</sup> -OH deformation

The IR spectra of 36 glycosides and their aglycones were studied. Glycosides were characterized by a doublet in the region 1099-1031 and 1066-1013  $\text{cm}^{-1}$ .<sup>3</sup>

Examination of the IR spectra of digitoxin revealed the presence of two polymorphs. One of the polymorphic crystals was obtained by recrystallization from 85% ethanol, and the other from the cold ethanol evaporation<sup>4</sup>.

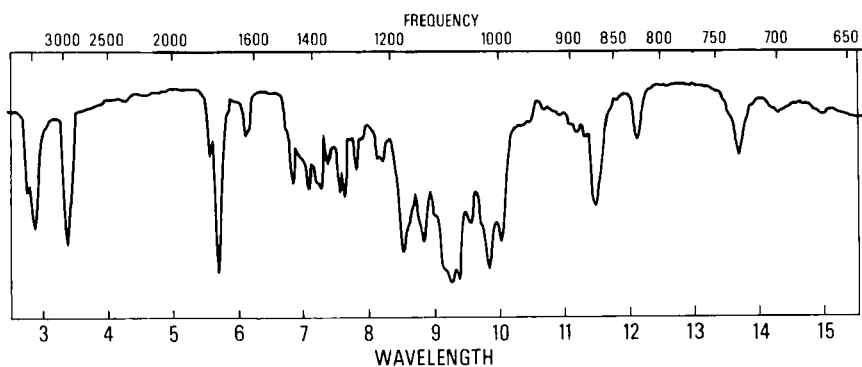
## 2.2 Nuclear Magnetic Resonance Spectrum

The NMR spectrum of digitoxin is complex even at 220 MHz, however 17 proton signals may be seen to low field of 2.5 ppm both in  $\text{CDCl}_3$  and  $\text{CD}_3\text{SOCD}_3$  after exchange with  $\text{D}_2\text{O}$  to remove the signals for OH. They may be assigned with reasonable certainty from chemical shift and coupling constants. The  $\delta$  values are listed as follows:

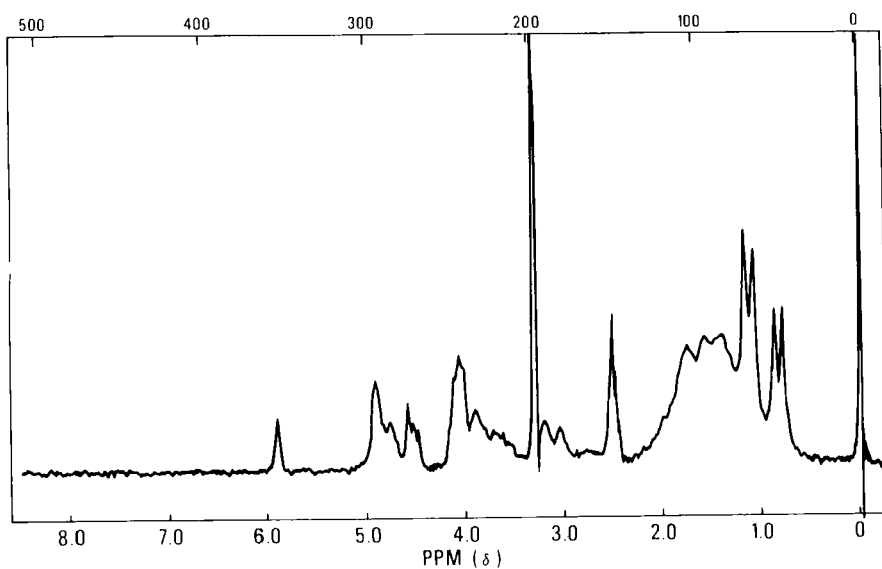
In  $\text{CDCl}_3$ : 2.79 ppm (broadened triplet), proton at 17; 3.20, 3.23, 3.27 (overlapping),  $J_{aa} = 10.0$  Hz,  $J_{ae} = 2.8$ , protons 4, 10', 16' (numbering from the anomeric proton away from the oxygen in each sugar ring sequentially starting with position 3 on the steroid); 3.77 unresolved protons 5', 11', 17'; 4.01, proton 3; 4.10, proton 15'; 4.22, unresolved protons 3' and 9'; 4.78, 4.97,  $J_{\text{gem}} = -18.0$  Hz,  $J_{21-22} = 1.8$ ,  $\text{CH}_2$  at 21; 4.84, 4.88, 4.91, (overlapping)  $J_{aa} = 10$ , protons 1', 7', 13'; 5.88, proton at 22.<sup>5</sup> See Fig.2.

Positions of the acetyl groups in partially acetylated cardenolides were established by the analysis of NMR spectra. Chemical shifts of protons belonging to specific acetyl groups differ sufficiently to serve as diagnostic characteristics.

DIGITOXIN



**Fig. 1** IR Spectrum of Digitoxin, USP Reference Standard. KBr Pellet. Instrument: Beckman IR-12 Spectrophotometer.



**Fig. 2** NMR Spectrum of Digitoxin, USP Reference Standard. Instrument: Varian T-60A.

The signals of the equatorial protons of digitoxose molecules are also of diagnostic value as their position changes when the adjacent axial group is acetylated<sup>6</sup>.

### 2.3 Ultraviolet Spectrum

The ultraviolet curve of a solution in methanol shows a peak at 218 nm,  $\epsilon$   $17.4 \times 10^{-3}$ .<sup>7</sup>

### 2.4 Mass Spectrum

Mass spectrometric data were obtained using electron impact and low resolution on a C.E.C. 110 mass spectrometer. The fragmentation pattern is typical of the steroid portion only. The highest significant mass is at m/e 357 which represents the digitoxigenin fragment.

### 2.5 Optical Rotation

Investigators have determined the optical rotation under different conditions:

$$[\alpha]_D^{20} = +4.8^{\circ} \quad (c=1.2 \text{ in dioxane})^8$$

$$[\alpha]_D^{20} = \text{about } +18^{\circ} \quad (c=2.5 \text{ in chloroform})^9$$

$$[\alpha]_{Hg}^{20} = \text{about } +21^{\circ} \quad (c=1.0 \text{ in chloroform})^9$$

$$[\alpha]_D^{20} = \text{about } +13^{\circ} \quad (c=1.0 \text{ in methanol})^7$$

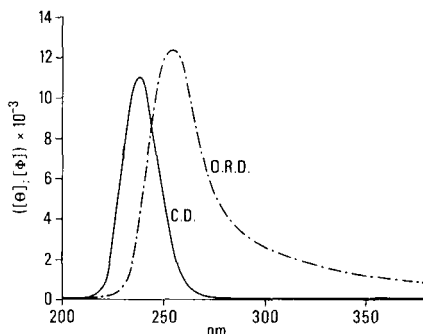
### 2.6 Optical Rotatory Dispersion (ORD) and Circular Dichroism (CD)

The circular dichroic (CD) spectra were recorded on a Cary 60 spectropolarimeter, equipped with Model 6002 CD unit. The ORD curve shows a positive  $[\alpha]$  of 13.1 and a positive Cotton effect of  $[\phi]$   $12.3 \times 10^{-3}$  at 254 nm as do many steroids. See Fig. 3. The CD curve shows a positive effect of  $[\phi]$   $11.1 \times 10^{-3}$  at 238 nm. These studies were done in methanol.<sup>7</sup>

## DIGITOXIN

Stereochemical effects are found in cardenolides with  $\alpha$ -ketol groups in the 11,12 position<sup>10</sup>.

*Fig. 3 ORD and CD Spectra of Digitoxin, USP Reference Standard. Instrument: Cary 60 Spectropolarimeter, equipped with Model 6002 CD Unit.*



### 2.7 Melting Range

The melting point of digitoxin is 256 - 257°C. (anhydrous).<sup>8</sup> Digitoxigenin has a melting point 250°C.<sup>11</sup>

The effect of protective environments such as a) immersing the substance under silicone oil, b) under an atmosphere of nitrogen and c) in an evacuated, sealed capillary tube were studied on a microscope hot stage. The melting points which are obtained under such conditions are often higher<sup>12</sup>.

### 2.8 X-Ray Diffraction Pattern

The X-ray diffraction pattern of digitoxin conforms to the following pattern<sup>13</sup>.

dA	I/I'	dA	I/I'	dA	I/I'
15	7	6.26	40	4.80	40
9.07	20	5.95	100	4.62	40
8.01	7	5.63	50	4.32	70
7.28	30	5.32	13	4.10	30
7.00	30	5.40	40	3.93	30

dA	I/I'	dA	I/I'	dA	I/I'
3.75	30	3.06	7	2.37	3
3.62	7	2.89	7	2.20	3
3.52	7	2.75	7	2.10	3
3.39	7	2.60	3	2.05	3
3.30	20	2.52	3	2.01	3
3.23	7	2.43	3	1.95	3

## 2.9 Polarography

A study of the polarographic characteristics of digitoxin in 50% ethanolic solution containing tetraethylammonium hydroxide as electrolyte, showed an average half-wave potential of -1.965 volts. The diffusion current wave height versus concentration graph indicated that quantities as low as 2 mcg could be determined. The method has been successfully applied to the tincture of digitalis<sup>14,15</sup>.

## 2.10 Solubility

Digitoxin is practically insoluble in water (1 g dissolves in about 100 liters at 20°C). One gram dissolves in about 40 ml chloroform, 60 ml ethanol, and 400 ml ethyl acetate. It is also soluble in ether, petroleum ether, benzene and vegetable oils.

## 3. SYNTHESIS

Digitoxigenin, a typical member of the cardenolide family has been synthesized using as the starting material methyl-3 $\beta$ -acetoxy-14 $\beta$ -hydroxy-5 $\beta$ -etinate by a seven-step sequence<sup>16</sup>. In the last step a solution of  $\alpha,\beta$ -unsaturated ester was treated with SeO<sub>2</sub> by boiling under reflux for 10 hours. The filtrate was poured into water and the product isolated with ether. Acid hydrolysis of digitoxigenin acetate yielded digitoxigenin (M.p. 246-249°C and  $[\alpha] +19^\circ$  in ethanol).

#### 4. STABILITY

The stability of two liquid extracts from the leaves of *Digitalis purpurea* was examined. Both products contained digitoxin and gitoxin. The activity of each drug was decreased by more than 10% of the initial value in less than three months at 20°C. The rate of decomposition was gitoxin > digitoxin<sup>17</sup>.

Storage of digitoxin preparations for one year did not significantly decrease their potency stored at temperatures up to 30°C. The potency was checked by Baljet colorimetric assay and by the biological method according to the Swedish Pharmacopeia XI<sup>18</sup>.

No breakdown of digitoxin in tablets, injections or solutions was found when stored for 5 years in the dark up to 30°C.<sup>19</sup>

#### 5. METABOLISM, PROTEIN BINDING AND CLINICAL ASSAY

##### 5.1 Metabolism

Digitoxin is completely absorbed following oral ingestion and its full effect appears as rapidly as by intravenous injection<sup>20</sup>. The liver is the main site of detoxification of digitoxin. It metabolizes very rapidly. One metabolite has been identified: digoxigenin-di-digitoxoside, produced by hydroxylation and the loss of one molecule of sugar.

Ten days after an injection, half of the dose is still present in the body, and some remains after 20 days. Following the administration of maintenance doses of 0.1 to 0.3 mg daily, 10% of the dose is excreted unchanged.

An experimental method has been developed in order to study the metabolic degradation of digitoxin and digoxin and changes in lipid solubility of the radioactive material in plasma<sup>21</sup>. The changes occur after the administration of radioactively labelled glycosides into an isolated perfused liver system (guinea pig) or to intact rabbits. The metabolic degradation of digitoxin and digoxin in the liver results in the formation of



the mono- and bis-digitoxosides of digoxigenin and of conjugated products. These metabolites are more polar than original glycosides. An increased plasma-chloroform coefficient indicates a change in the ratio of polar/ nonpolar substances in the extracted medium.

## 5.2 Protein Binding

The protein binding of digitoxin is thought to account for the higher plasma levels. The results were obtained by the Rb<sup>86</sup> uptake inhibition technique, suggesting its probable value as a clinically applicable quantitative method for the detection of commonly used digitalis glycosides in plasma. Comparison results by two laboratories were presented<sup>22</sup>.

The protein binding capacity of poorly soluble cardenolides in water is determined from the saturation concentration of these substances both in protein solution and in their ultrafiltrate in microscale. Figures about the binding of digitoxin, digoxin etc. to human serum protein, and the binding of digitoxin to serum proteins of different species, are presented. The cardenolide-serum protein binding is affected by calcium ions<sup>23</sup>.

## 5.3 Determination in Blood

### 5.3.1 Chemical Methods

Digitoxin concentration in the blood of orally digitalized patients was quantitatively determined employing a combination of TLC and a fluorometric method:

30 ml ~~veinous blood~~ was diluted to 300 ml with water, and the haemolysate was extracted with chloroform. The chloroform extract was evaporated under mild condition (temp. not exceeding 40°C.). The residue was dissolved in 50% aqueous methanol and then extracted with petroleum ether. Remaining methanol was extracted with chloroform. The residue after chloroform evaporation (redissolved in an exact amount of chloroform) was applied to Kieselgel G plates. Mobil phase: methylenchloride/isopropanol/formamide, 80:19:1. Spray reagent:

a mixture of chloramine and trichloroacetic acid. After spraying the plates were heated at  $115^{\circ}\text{C}$ . for 10 min. Optimal fluorescence in UV light was at  $365\text{ nm}^{24}$ .

Digitoxin in blood plasma was determined by enzyme p-esterhydrolase and ATP-ase inhibition technique<sup>25</sup>.

### 5.3.2 Physical Methods

Cardiac glycosides can be determined in biological fluids from concentrations of 1 ng/ml by their inhibition of the uptake of Rb by red blood cells. The glycoside extract was incubated at  $37^{\circ}\text{C}$ . for 2 hours with dimethyl sulfoxide, red blood cells, and a solution of RbCl. The Rb remaining in the supernatant is measured by atomic absorption spectrometry<sup>26</sup>.

### 5.3.3 Radioimmunoassay (Deuterium and Tritium Labeled Digitoxin)

All three protons in the unsaturated butenolide ring can be exchanged in a base-catalyzed process. The exchange takes place even under very mild conditions and the ring does not open. Digitoxin was treated with triethylamine and deuterium oxide, and UV, IR and NMR data showed that the compound formed corresponds to the 21,21,22-trideuterodigitoxin. Similar reaction takes place with tritium oxide. The exchange is limited to the three protons in the unsaturated butenolide ring<sup>27</sup>.

Clinically applicable radioimmunoassay techniques for measurement of serum digitoxin have been used to determine levels of this drug from 250 patients. Unlabeled drug in the patients' serum displaces tritiated digitoxin (added in vitro) from specific antibody binding sites. The procedure requires one hour<sup>28</sup>.

The pharmacodynamics of digitoxin in man have been studied utilizing a sensitive (0.2 ng/ml) specific radioimmunoassay. Patients receiving 0.1 mg of digitoxin daily had a mean serum digitoxin level of 25 ng/ml, and 44 ng/ml was detected in patients receiving 0.2 mg daily<sup>29</sup>.

In another study unlabeled digitoxin in the unknown sample competes with a tritiated digitoxin tracer for binding sites of high affinity rabbit antibodies to a human serum albumen-digoxin conjugate. Free labeled digitoxin was separated from the antibody-bound fraction by adsorption to dextran-coated charcoal. The method is sensitive to 2 ng/ml or less<sup>30</sup>.

## 6. METHODS OF ANALYSIS

### 6.1 Identification Tests

Dissolve about 1 mg of digitoxin in 2 ml of a solution prepared by mixing 0.3 ml of a 9% aqueous ferric chloride solution and 50 ml of glacial acetic acid, and underlay with 2 ml of sulfuric acid: at the zone of contact of the two liquids a brown color is produced, and it gradually changes to light green, then to blue, and finally the entire acetic layer acquires a blue color<sup>31</sup>.

Dissolve about 0.2 mg of digitoxin in 2 ml of a freshly prepared 1 in 100 solution of m-dinitrobenzene in ethanol, and allow to stand for 10 min. with frequent shaking. Add 2 ml of a mixture of 1 volume of a 10% tetramethylammonium hydroxide and 200 volumes of ethanol, and mix: a red-violet color develops slowly and then fades<sup>31</sup>.

### 6.2 Elemental Analysis

Elemental analysis of digitoxin as  $C_{41}H_{64}O_{13}$ :

C - 64.4%

H - 8.4%

O - 27.2%

### 6.3 Chromatography

#### 6.3.1 Column Chromatography

Aluminum oxide, siliceous earth, and Sephadex are adsorbents commonly used for the separation of cardenolide glycosides and their metabolites.

The USP XVIII employs a column of siliceous earth previously cleaned with hydrochloric acid, and then activated at 500°C. Formamide is added as the stationary phase. Digitoxin is eluted with a mixture of benzene/chloroform, 3:1.

Aluminum oxide deactivated with 3% water and packed in a column of 1.5 cm diameter to a height of 10 cm has been used successfully for the separation of digitoxin from its metabolites. Elution is achieved with 100 ml of chloroform followed by 35 ml of 2% ethanol in chloroform, and finally with 250 ml of 10% ethanol in chloroform. Digitoxin and its metabolites are found in that portion of eluate between 275 and 425 ml (this includes the chloroform prewash)<sup>32</sup>.

Sephadex G-200, swelled with a mixture of water/methanol, 7:3, was employed for the separation of digitoxin and its metabolites. Sephadex was packed in a column of 1 cm diameter to a height of 15 cm. The sample was eluted using the above mixture. Cardenolides were in the fraction between 10 and 25 ml<sup>32</sup>.

#### 6.3.2 Thin Layer Chromatography

A rapid separation of digitoxin from digoxin and acetyldigitoxin can be achieved applying 2  $\mu$ l of a 0.01% sample solution in chloroform/methanol, 1:1 to Kieselgel G plates. As the eluent a chloroform/methanol, 9:1 mixture was used. Detecting agent: hydrochloric acid. The spots of digitoxin ( $R_f$  0.33), digoxin ( $R_f$  0.24) and acetyldigitoxin ( $R_f$  0.48) were dark brown after 5 min. Drying at 110°C. for 5 min and examination under ultraviolet light (365 nm) showed brown spots for digitoxin and acetyldigitoxin, while digoxin spot was blue. The limit of detection is 0.05 mcg<sup>33</sup>.

Digitoxin was separated from digoxin on Silica Gel G plates with chloroform/methanol, 88:12 developing solvent. The zones were located by spraying with 1% iodine in chloroform, then removed from the plate and extracted with chloroform/methanol, 1:1 mixture. After centrifugation, a 7 ml aliquot of supernatant was evap-

orated to dryness. The residue was dried and then treated with dioxanthylurea reagent and the chromophore read at 535 nm<sup>34, 35</sup>.

A method for the direct quantitative evaluation of digitoxin, digoxin and acetyldigitoxin on TLC using spectrofluorometry was investigated. The only reagent used was hydrochloric acid. Linear standard curves were obtained when the area under the fluorometric curve was correlated with the amount of glycosides applied. The optimal range for the spectrofluorometric determination of these three glycosides was about 0.25 mcg<sup>36</sup>.

Separation of the cardiac glycosides digitoxin and digoxin from their 20,22-dihydro derivatives can be achieved by multiple TLC on cellulose films<sup>37</sup>.

An ultramicro fluorescent spray reagent for detection and quantitation of digitoxin and other cardiotonic glycosides on TLC was described. The spray reagent consists of ascorbic acid, methanol, hydrochloric acid and hydrogen peroxide. The limits of detection were 0.01 mcg<sup>38</sup>.

Application of diffusion and fluorescence to the direct determination of digitoxin was achieved by converting digitoxin into a fluorescent derivative by means of a reagent containing p-toluenesulfonic acid, hydrochloric acid, ascorbic acid and hydrogen peroxide. Sensitivity: 0.3-1 mcg<sup>39</sup>.

A TLC system on silica gel G plates has been developed using as the mobile solvent a mixture of methylene chloride/methanol/formamide, 80:19:1, and spraying the plates with acid-ferric chloride<sup>31</sup>. The same technique can be used for the identification of digitoxin, digoxin and acetyldigitoxin, and for the determination of any glycoside present in their drug formulations. Digoxin is not activated to visible fluorescence at room temperature by acid-ferric chloride reagent. Therefore any fluorescence present immediately after spraying is due to glycoside alone. Heating the plate at 100°C. destroys the glycoside fluorescence and converts the digoxin to a fluo-

rescent anhydro derivative which may be seen under both UV and visible light<sup>40</sup>.

### 6.3.3 Paper Chromatography

After separation by paper chromatography in formamide saturated methylethylketone/xylene, 1:1 mixture, digitoxin and digoxin were determined with xanthidrol (10-fold excess of reagent in acetic acid/hydrochloric acid, 99:1 mixture). It was necessary to heat the reaction mixture for 20 min. at 60°C. A 1:1 complex ( $\lambda_{\max}$  535 nm), stable for 3 hours was formed. Beer's law was obeyed over the range 1-20 mcg/ml<sup>41, 42</sup>.

A butenolide ring specific method of quantitative paper chromatographic analysis of digitoxin using 2,4,2',4'-tetranitrodiphenyl is described. Paper: Schleicher and Schüll 2043b impregnated with formamide. Developing solvent: methylethylketone/xylene, 1:1 saturated with formamide. The chromatogram was dried for 15 min. at 60°C.<sup>43</sup>

### 6.3.4 Gas Chromatography

Trimethylsilyl ether derivatives of digitoxin, digoxin and gitoxin have been shown to be resolvable on a gas chromatographic column packing containing as a liquid phase 2.5% OV-1 or OV-17 on Chromosorb W. Gas chromatography was performed on a Barber-Colman 5000 series instrument equipped with hydrogen flame detector. During isothermal operation injection port and column bath temperatures were identical. Detector temperature was maintained at 340°C. In the case of temperature programming, injection port temperature was identical to the starting temperature: 240°C.<sup>44</sup>

An improved method for the gas chromatographic identification of digitalis cardenolides as their anhydro derivatives has been developed, resulting in greatly reduced retention times and enhanced resolution. Retention data of 18 cardenolides on three liquid phases are reported. Spectral evidence is presented showing that the tertiary 14 $\beta$ -OH group is neither affected by esterification nor etherification<sup>45</sup>.

### 6.3.5 High Speed Liquid Chromatography

High speed liquid chromatography has been used to examine steroids and steroid conjugates such as digitoxin or digoxin. In these studies reverse phase liquid partition chromatography was employed. The columns consisted of a cyanoethylsilicone polymer (Dupont Zipax<sup>R</sup> ANH) and the mobile phase was a mixture containing 2.5% methanol and 97.5% water. The compounds were detected by means of a 254 nm photometer<sup>46</sup>.

### 6.4 Colorimetric Analysis

The methods for the determination of cardiac glycosides can be divided into three general groups based on: 1- the sugar moiety, 2- the butenolide moiety, and 3- the steroid part of the molecule.

1- As far back as 1885, a colorimetric method<sup>47</sup> was published using equal amounts of sulfuric acid and ethanol with the addition of ferric chloride. Others<sup>48</sup> used a solution of ferric sulfate in concentrated sulfuric acid, or added ferric chloride to a solution of glycoside in acetic acid and then underlaid the Kiliani reagent<sup>49</sup>. Many methods employ xanthydrol as the reagent for digitoxose<sup>50</sup>.

2- The reagent employing picric acid in alkaline ethanol is the most frequently used<sup>51</sup>. There are many modifications and applications of this reaction<sup>52, 53, 54, 55, 31</sup>.

The official USP XVIII method depends on a chromatographic separation on siliceous earth in the presence of formamide, and a reaction in the butenolide side chain by picric acid in alkaline solution.

The application of m-dinitrobenzene for the quantitative determination of cardiac glycosides is very successful<sup>56</sup>, as well as 1,3,5-trinitrobenzene in alkaline medium<sup>57</sup>. Some authors use 2,4-dinitrodiphenylsulfone in alkaline ethanol<sup>58</sup>. The other reagents used are 2-naphthoquinone-4-sulfonate<sup>59</sup>, and 2,2',4,4'-tetranitrodiphenyl<sup>60, 61</sup>.

The reagents based upon the reaction in butenolide ring have a wide application in cardiac glycosides metabolism studies. These reagents react with any glycoside or its metabolite that still contains the intact butenolide ring.

3- Methods based upon the reaction in the steroid moiety are for the most part fluorometric.

### 6.5 Fluorometric Analysis

Methods based upon the reaction in the steroid moiety are mainly dehydration type of reactions such as that using syrupy phosphoric acid<sup>62</sup>, or equal amounts of hydrochloric acid and glycerol as the dehydrating agents<sup>63</sup>. Hydrogen peroxide, hydrochloric acid and methanol<sup>64, 65</sup>, or a mixture of sulfuric and phosphoric acids with the addition of ferric chloride<sup>66</sup> are also used.

The fluorophor obtained with a mixture of acetic anhydride, acetyl chloride and trifluoroacetic acid, supports the theory, based on NMR, IR and fluorescence activation spectral data, that a low yield of a highly conjugated fluorophor of substituted 3,4-benzpyrene is obtained<sup>67</sup>.

### 6.6 Electrophoresis

Digitoxin may be detected and estimated in human autopsy tissues by paper electrophoresis according to an author<sup>68</sup> who used a mixture of oxalic acid, boric acid and ethanol to develop different colors depending upon the compound. The limit for identification was about 15 mcg for digitoxin, and 10 mcg for digitoxigenin. The digitoxin was totally degraded in the tissues following putrefaction for three months<sup>69</sup>.

### 6.7 Automated Assay

An automated procedure using a standard Technicon automatic analyzer system is described for the unit dose analysis of digitoxin and digoxin in tablets<sup>70</sup>. The technique is based on the fluorometric measurement of the dehydration products of the cardiotonic steroids resulting from their reaction with hydrogen peroxide and hydro-



chloric acid. The automated system as described is capable of analyzing 12 tablets per hour.

## 7. CLEAVAGE OF CARDIAC GLYCOSIDES

The isolation of cardiac aglycones from plant material is made difficult by the lack of reliable methods that will maintain the genins intact after hydrolysis of their glycosides.

A procedure is described for cleaving the sugar bond of digitoxin in organic media and under mild acid conditions. Maximum yields were obtained in 30 min. of reaction time at 50°C; in a medium consisting of anhydrous tetrahydrofuran made 0.002 N with respect to perchloric acid. Digitoxigenin is stable under these conditions<sup>71</sup>.

## 8. BIOLOGICAL ACTIVITY

### 8.1 Characteristic Structural Features

From the studies of biological activity<sup>72</sup> four characteristic structural features of the genins can be easily identified as essential for cardiac activity:

1- The Lactone Ring: The double bond of the lactone ring is apparently necessary for cardiac action. The rupture of the lactone ring results in a loss of cardiac action.

2- The Hydroxy Group on C-14 Atom: This hydroxy group is important and its modification results in a significant loss of activity. If this group is removed, the important stereochemical relationship is destroyed so that loss of activity could be due either to the loss of the 14-hydroxyl group, or to the alteration of the cis C/D ring arrangement<sup>73</sup>.

3- Sugars at C-3 Atom: In the case of the aglycone, the attachment of one or more sugars at C-3 usually results in increased activity.

4- Stereochemical Arrangements: A "cis" fusion of the C and D rings is necessary for activity. Other types of natural steroids have the "trans" configuration. The presence of two hydroxyl groups at C-12 and C-16 in the molecule of di-

gitoxigenin diminishes the activity by two-thirds. 14 $\alpha$ ,15 $\alpha$ -epoxy-14-anhydrodigitoxigenin is practically inactive.

## 8.2 Bioassay

Experiments were made to examine the suitability of young chicks for the assay of digitalis glycosides. The jugular vein was cannulated and a volume of the test solution was infused. The dose was repeated at 5 min. intervals until cardiac arrest was noticed. The order of susceptibility of tested animals was as follows: pigeon > chick > rat<sup>74</sup>.

## 9. ACKNOWLEDGMENT

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THE LITERATURE SEARCH WAS CONDUCTED UP TO MAY 1973.

# **DIPHENHYDRAMINE HYDROCHLORIDE**

*Ira J. Holcomb and Salvatore A. Fusari*

1. Description

- 1.1 Name, Formula, Molecular Weight
- 1.2 Appearance, Color, Odor

2. Physical Properties

- 2.1 Infrared Spectrum
- 2.2 Nuclear Magnetic Resonance
- 2.3 Ultraviolet Spectrum
- 2.4 Mass Spectrum
- 2.5 Optical Rotation
- 2.6 Melting Range
- 2.7 Differential Thermal Analysis
- 2.8 Solubility
- 2.9 Crystal Properties
  - 2.91 Optical Crystal Properties
  - 2.92 X-Ray Diffraction
- 2.10 Distribution Coefficients
- 2.11 Aggregation: Micelle Formation
- 2.12  $pK'_a$  values
- 2.13 Metal Complex Formation and Binding

3. Synthesis

4. Stability - Degradation

5. Drug Metabolic Products - Pharmacokinetics

6. Identification: Microchemical Tests

7. Methods of Analysis

- 7.1 Elemental Analysis
- 7.2 Spectrophotometric Analysis

DIPHENHYDRAMINE HYDROCHLORIDE

- 7.21 Elemental Analysis
- 7.22 Separation Methods Prior to Spectrophotometric Assay
- 7.23 Methods Based on Conversion to Benzophenone Prior to Spectrophotometric Assay
- 7.24 Method Based on Conversion to Chloranilic Acid Prior to Spectrophotometric Assay
- 7.3 Colorimetric Analysis
  - 7.31 Ion-pair Extraction Methods
  - 7.32 Ammonium Reineckate Methods
  - 7.33 Picric Acid Method
  - 7.34 Method Based on Molle Reaction
  - 7.35 Miscellaneous Colorimetric Methods
- 7.4 Titrimetric Analysis
  - 7.41 Direct Methods of Titration
  - 7.42 Separation Prior to Titration
    - 7.421 Reineckate Salt Formation
    - 7.422 Complexometric Method
    - 7.423 Slurry Method
    - 7.424 Ion Exchange Method
    - 7.425 Extraction Method
  - 7.43 Miscellaneous Titrimetric Methods
- 7.5 Fluorometric Analysis
- 7.6 Automated Analysis
- 7.7 Biological Assay
- 7.8 Gravimetric Analysis
- 7.9 Chromatography
  - 7.91 Paper Chromatography
  - 7.92 Thin Layer Chromatography
  - 7.93 Gas Chromatography



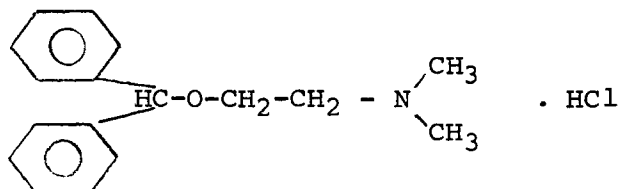
- 7.931 Direct Methods on  
Neutral Columns
- 7.932 Direct Methods on  
Basic Columns
- 7.933 Oxidation to Benzophenone  
Prior to Gas Chromato-  
graphy
- 7.94 Column Chromatography
- 7.95 Electrophoresis

## 8.0 References

1. Description1.1 Name, Formula, Molecular Weight

Diphenhydramine hydrochloride is 2-(diphenylmethoxy)-N,N-dimethylethylamine hydrochloride.<sup>1</sup> Six additional chemical names are listed in The Merck Index<sup>2</sup>, along with twelve trade names. One additional name is Dimedrol.

The empirical formula is  $C_{17}H_{21}NO \cdot HCl$  with a molecular weight of 291.82.



The CAS Registry Number is 58-73-1 for 2-(diphenylmethoxy)-N,N-dimethylethylamine and for the hydrochloride, 147-24-0.

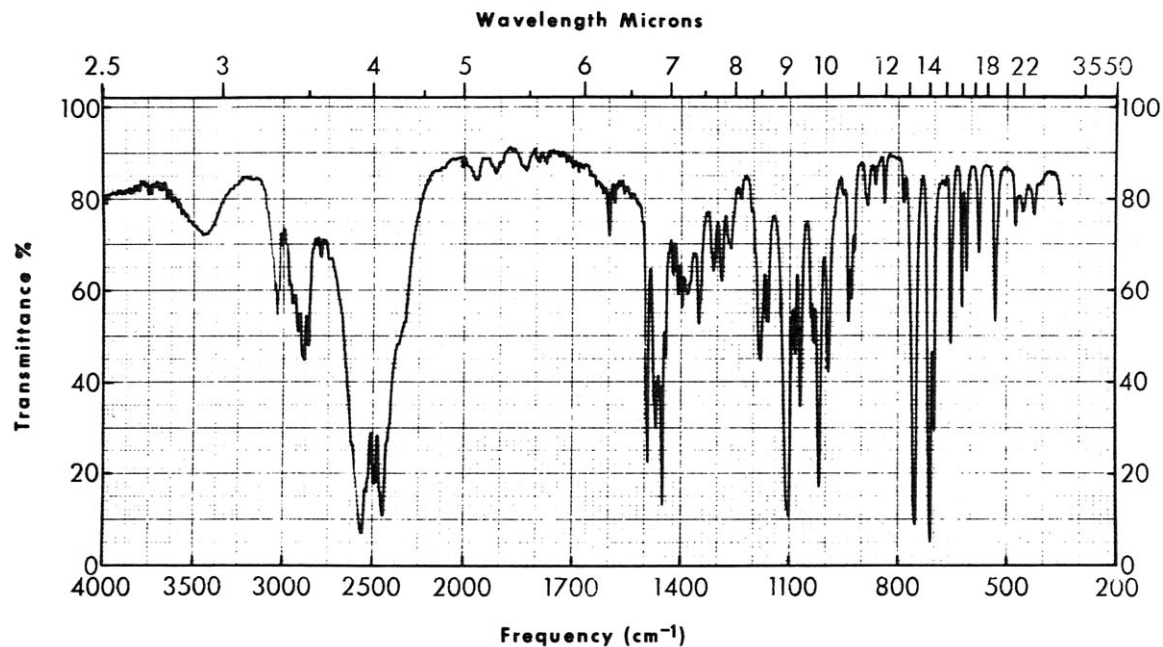
1.2 Appearance, Color, Odor

White, odorless, crystalline powder.<sup>1</sup>

2. Physical Properties2.1 Infrared Spectrum

The infrared spectrum of diphenhydramine hydrochloride is presented in Figure 1. The Sadtler Reference Number is 9382. The spectrum is used for control purposes.<sup>1,3</sup> Spectra are presented by de Roos<sup>3,4</sup> and Wallace.<sup>5</sup>

The infrared band assignments are given in Table I.



**Fig. 1. Infrared Spectrum of Diphenhydramine Hydrochloride, U.S.P. Parke-Davis & Co.  
Lot No. 563463. Instrument: Perkin-Elmer 621, Phase: KBr, 1:300.**

Table I. Infrared Band Assignments<sup>6</sup>  
for Diphenhydramine Hydrochloride

<u>Wave No. (cm<sup>-1</sup>)</u>	<u>Assignment</u>
3000 - 3100	aromatic CH stretch (3035)
2400 - 2700	-N(CH <sub>3</sub> ) <sub>2</sub> ·HCl
1600, 1587, 1498, 1460	aromatic ring skeletal vibrations
1472	CH <sub>2</sub> bending
1384	CH <sub>3</sub> bending
1119	C-O-C stretching
717, 760	C-H out of plane deformation of mono-substituted phenyl

## 2.2 Nuclear Magnetic Resonance

In Figure 2 the nuclear magnetic resonance spectrum of diphenhydramine hydrochloride is presented. The spectral peak assignments<sup>7</sup> are presented in Table II. The Sadtler NMR Reference Number is 14360.

## 2.3 Ultraviolet Spectrum

The ultraviolet spectrum of diphenhydramine hydrochloride is presented in Figure 3. The absorptivities at 258 nm. listed in Table III compare well with the literature values of 15.4<sup>8</sup> in methanol and 16.5<sup>9</sup> in an aqueous system.

## 2.4 Mass Spectrum, Low Resolution<sup>11</sup>

A plot of the relative intensities vs. mass/charge ratio is presented in Figure 4 and summarized in Table IV. The ionization potential is 70 electron volts.

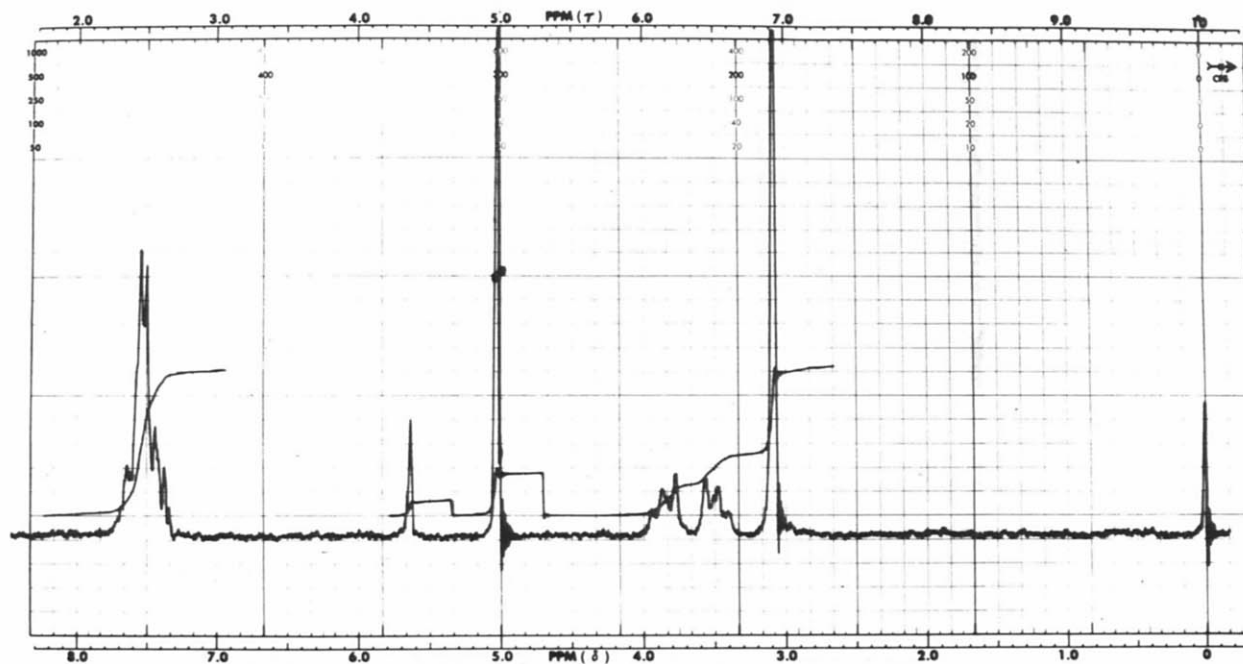
## 2.5 Optical Rotation

Diphenhydramine hydrochloride is not optically active.

## 2.6 Melting Range

Diphenhydramine hydrochloride melts in the range 167° to 172°C.<sup>1</sup> The actual range in which the compound melts is usually less than 2°C. The melting point is affected by the rate of heating<sup>12</sup> as shown in Table V.

Data was obtained from melting point scans using the Mettler FP-1.

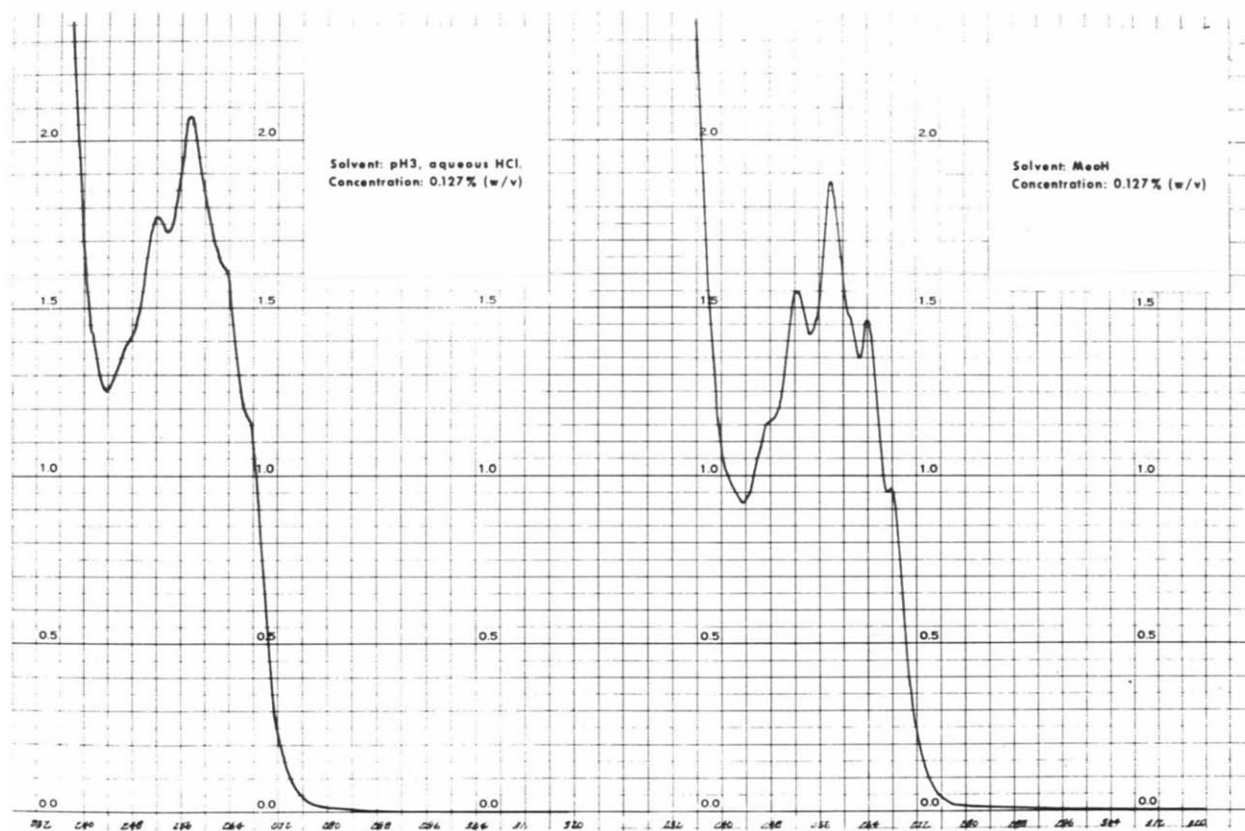


**Fig. 2. Nuclear Magnetic Resonance Spectrum of Diphenhydramine Hydrochloride, U.S.P., Parke-Davis & Co. Lot No. 563463.**

**Instrument: Varian A-60. Solvent. D<sub>2</sub>O. Sweep Offset: 0 cps.**

Table II. NMR Spectral Assignments for  
Diphenhydramine Hydrochloride

<u>Chemical Shift (ppm.)</u>	<u>Proton Assignment</u>
3.10	$\text{N} \begin{array}{l} \text{CH}_3 \\ \text{CH}_3 \end{array}$
3.55	$\text{N}-\text{CH}_2$
3.85	$\text{O}-\text{CH}_2$
5.0	$\text{H Cl}$
5.65	$\phi \text{CH} -$
7.3 - 7.7	Aromatic Ring Protons



**Fig. 3. Ultraviolet Spectrum of Diphenhydramine Hydrochloride, U.S.P., Parke-Davis & Co. Lot No. 563463. Instrument: Cary 14.**



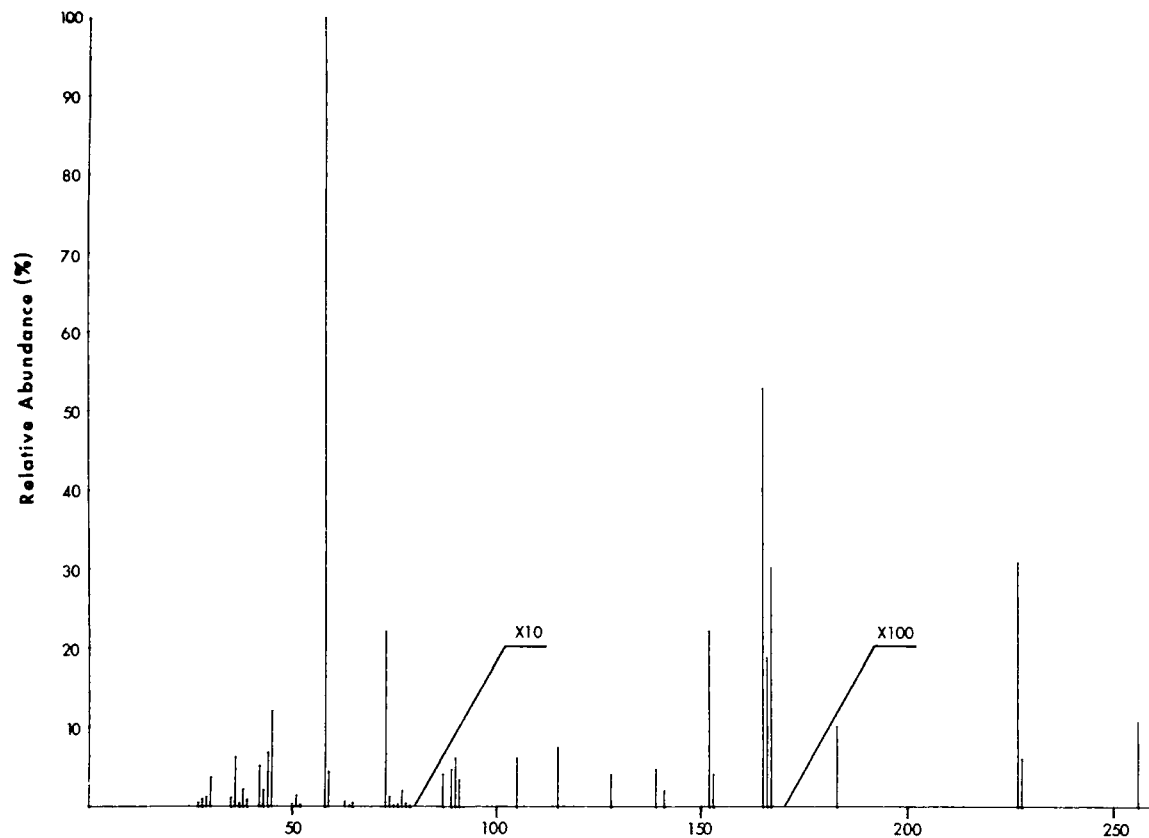
Table III. Absorptivities<sup>10</sup> of  
Diphenhydramine Hydrochloride,  
Parke, Davis & Co., Lot No. 563463

Aqueous Medium (pH 3):

<u>Wavelength</u>	<u>a (1%, 1 cm.)</u>	<u><math>\epsilon</math></u>
267 nm(s)	9.3	270
263 nm(s)	12.7	370
257.5 nm(s)	16.3	476
252 nm	13.95	406

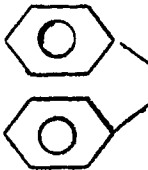
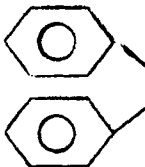
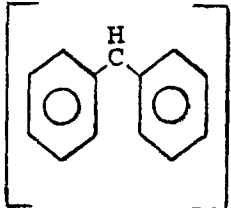
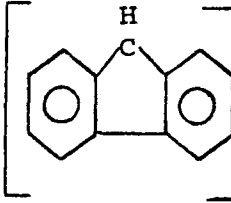
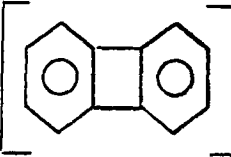
Methanol, absolute:

268 nm	7.95	232
264 nm	12.1	353
258 nm	15.5	452
252 nm	12.8	374



**Fig. 4. Mass Spectrum of Diphenhydramine Hydrochloride, U.S.P., Parke-Davis & Co. Lot No. 563463.**  
**Instrument: Finnigan Quadrupole Mass Spectrometer, Model 1015.**

Table IV  
Low Resolution Mass Spectrum Assignments  
for Diphenhydramine Hydrochloride

<u>Measured Mass</u>	<u>Relative Intensity</u>	<u>Structural Assignments</u>
256	10.67	 $\text{CH-O-CH}_2\text{-CH}_2\text{-N}^+\begin{matrix} \text{H} \\ \text{CH}_3 \end{matrix}$ $\text{C}_{17}\text{H}_{22}\text{NO}$ (M+1)
183	10.0	 $\text{H}^+ - \text{C} - \text{O}$ $\text{C}_{13}\text{H}_{11}\text{O}^+$
167	30.0	 $\text{C}_{13}\text{H}_{11}^+$
165	52.67	 $\text{C}_{13}\text{H}_9^+$
152	22.00	 $\text{C}_{12}\text{H}_8^+$

DIPHENHYDRAMINE HYDROCHLORIDE

Table IV(cont.)

<u>Measured Mass</u>	<u>Relative Intensity</u>	<u>Structural Assignments</u>
73	22.00	$\left[ \text{C}_2\text{H}_5-\text{N} \begin{array}{l} \nearrow \text{CH}_3 \\ \searrow \text{CH}_3 \end{array} \right]^+ \quad \text{C}_4\text{H}_{11}\text{N}$
58	100	$\text{CH}_2=\text{N}^+ \begin{array}{l} \nearrow \text{CH}_3 \\ \searrow \text{CH}_3 \end{array} \quad \text{C}_3\text{H}_8\text{N}$
45	12	$+ \text{N}^+ \begin{array}{l} \text{H} \\   \\ \nearrow \text{CH}_3 \\ \searrow \text{CH}_3 \end{array} \quad \text{C}_2\text{H}_7\text{N}$

Table V. Melting Point and Range<sup>12</sup> of Diphenhydramine

Hydrochloride, Parke, Davis & Co., Lot No. 563463

<u>Start Temp. (°C.)</u>	<u>Heating Rate</u>	<u>Range</u>	<u>Mid-Point</u>
163°	1°C/min.	167.7-168.6 (0.9)	168.1
		167.7-168.7 (1.0)	168.2
158°	3°C/min.	168.7-169.3 (0.6)	169.0
		168.7-170.0 (1.3)	169.4

## 2.7 Differential Thermal Analysis

The DTA curve obtained using a Mettler TA 2000 is shown in Figure 5. The percent purity found for the sample, Diphenhydramine Hydrochloride, USP, Parke, Davis & Co., Lot No. 593125, is 99.62%.<sup>13</sup>

## 2.8 Solubility

The solubility of diphenhydramine in water is 0.7 mg./ml.<sup>10</sup> Solubilities of diphenhydramine hydrochloride have been determined<sup>14</sup> and are presented in Table VI.

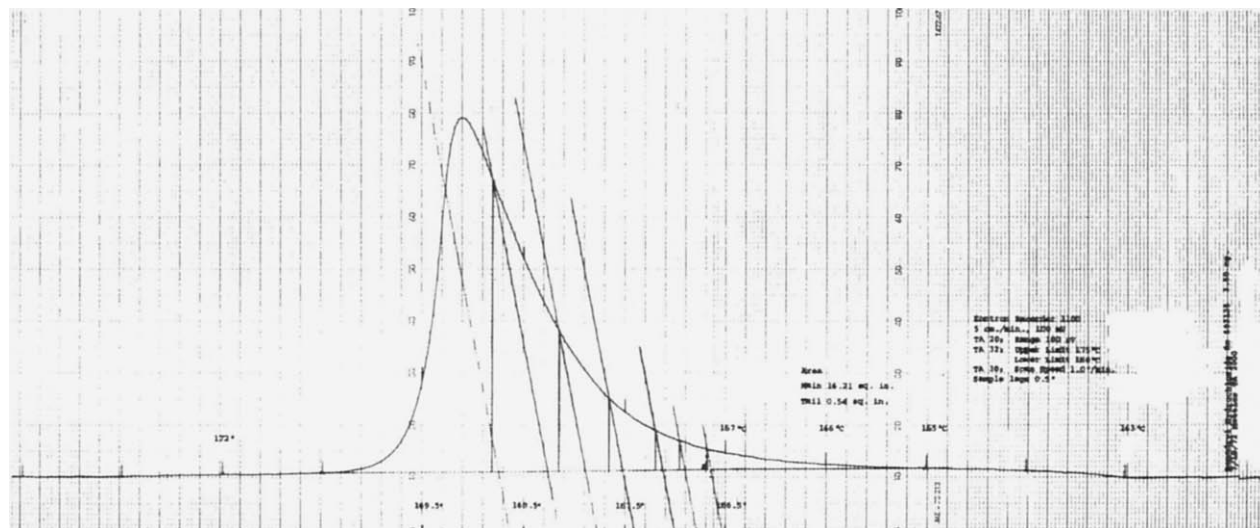
## 2.9 Crystal Properties

The optical crystallographic constants have been reported by Keenan.<sup>15</sup> Diphenhydramine hydrochloride is described as colorless, mostly six-sided plates with lengthwise cleavage. The  $n^{20}_D$  values are:  $\alpha$ , 1.602;  $\beta$ , 1.625; and  $\gamma$ , 1.630; all  $\pm 0.002$ . In parallel polarized light, extinction is parallel and the sign of elongation is negative. Shell<sup>16</sup> also reported on optical crystallographic properties and gave the density as 1.189.

## 2.92 X-Ray Diffraction

The X-Ray diffraction data for diphenhydramine hydrochloride were reported by Gadret<sup>17</sup>. The compound has been run by Krc<sup>18</sup> and the diffraction pattern is presented in Figure 6.

The calculated "d" spacings<sup>18</sup> for the diffraction pattern are given in Table VII. The  $2\theta$  angles were corrected on the diffraction pattern using known values for calcite added to a sample.



**Fig. 5 Diphenhydramine Hydrochloride, D.T.A Curve, Parke-Davis & Co. Lot No. 593125.**  
**Instrument: Mettler TA 2000  $\Delta H=7.495$  kcal. Melting point: 168.3°C.**

Table VI. Solubility<sup>14</sup> of Diphenhydramine  
Hydrochloride in Various Solvents

<u>Solvent</u>	<u>Solubility,</u> <u>mg./ml.</u>
Water	858
Methanol	599
Alcohol, 95%	408
Chloroform	394
Isopropyl Alcohol	35
Acetone	16



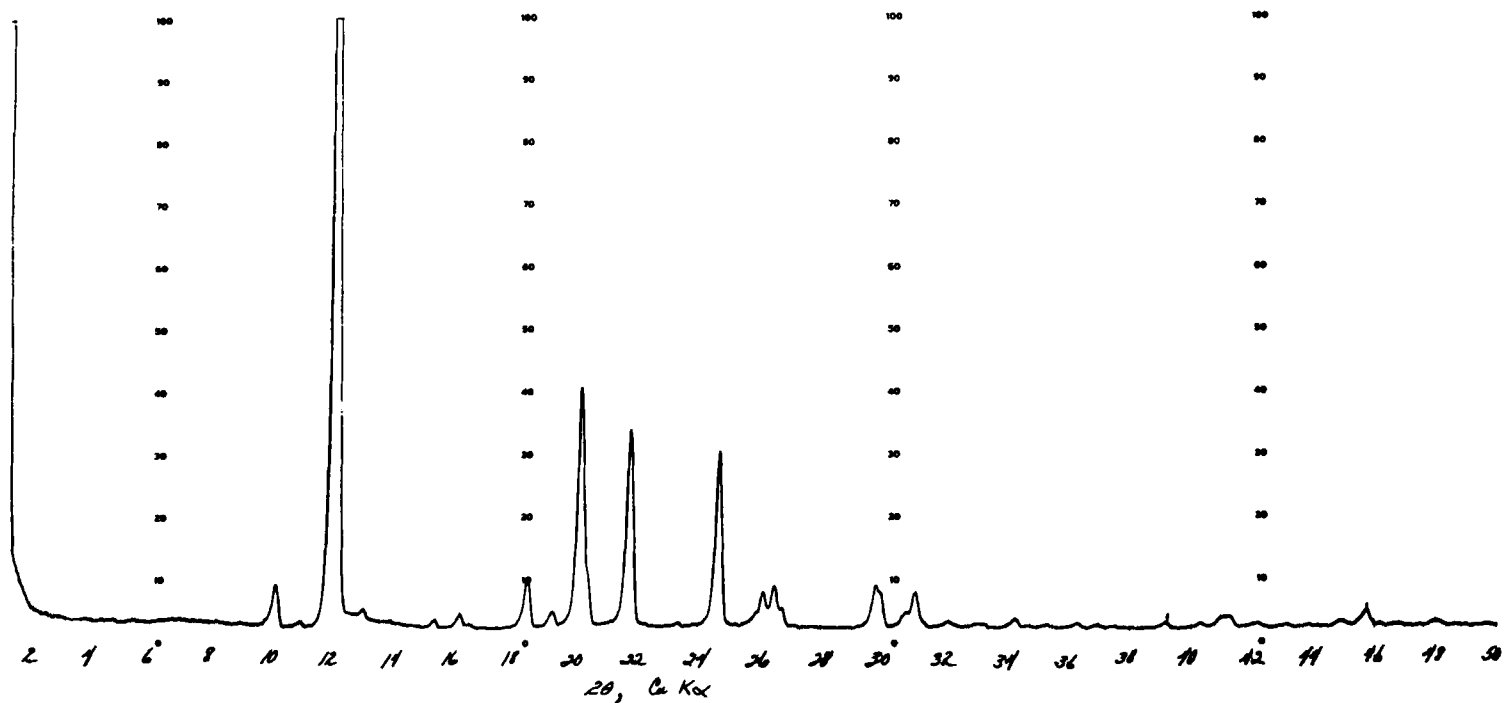


Fig. 6. X-Ray Diffraction pattern of Diphenhydramine Hydrochloride, U.S.P., Parke-Davis & Co.

Lot. No. 563463. Instrument: Norelco Diffractometer. Radiation:  $\text{Cu K}\alpha$

DIPHENHYDRAMINE HYDROCHLORIDE

Table VII. Diphenhydramine Hydrochloride:  
Calculated "d" Spacings and I/I<sub>1</sub> Values

Radiation: CuK<sub>α</sub>, λ 1.5418

Filter: Ni

<u>d(A°)</u>	<u>I/I<sub>1</sub></u>	<u>d(A°)</u>	<u>I/I<sub>1</sub></u>
8.56	7	2.86	4
7.97	<1	2.78	1
7.19	100	2.75	<1
6.74	2	2.70	1
5.70	<1	2.60	2
5.42	5	2.58	<1
5.30	<1	2.53	<1
4.78	13	2.46	<1
4.59	5	2.42	<1
4.37	20	2.29	1
4.05	24	2.19	1
3.85	2	1.98	1
3.59	12		
3.40	7		
3.35	11	d	7.19 4.05 4.37 8.56
3.33	6	I/I <sub>1</sub>	100 24 20 7
2.98	4		
2.91	3		

## 2.10 Distribution Coefficients

Doyle<sup>19</sup> has determined the distribution behavior of diphenhydramine hydrochloride in chloroform:water and ether:water. A logarithmic distribution diagram is presented in the study for selection of partition chromatographic systems.<sup>19</sup> The distribution of diphenhydramine hydrochloride between chloroform:water as a function of aqueous p-toluenesulfonic acid was also studied by Doyle.<sup>20</sup> The solvent composition effect on the partition of amines, in general, has been studied.<sup>21</sup>

Konyushko<sup>22</sup> examined the effect of pH on the distribution of diphenhydramine between water and chloroform. The extraction of diphenhydramine with chloroform in presence of  $\text{NH}_4\text{F}$ ,  $\text{KF}$ ,  $\text{KCl}$ ,  $\text{KBr}$ ,  $\text{KI}$  and  $\text{KSCN}$  as salting out agents at  $20^\circ$  and pH 3 has been reported.<sup>23</sup>

## 2.11 Aggregation - Micelle Formation

Diphenhydramine hydrochloride forms aggregates in solution.<sup>24</sup> Attwood<sup>25</sup> has determined the critical micelle concentration using scattering at an angle of  $90^\circ$  to the incident beam and determining the inflection points in the plot versus the molal concentration.

## 2.12 $\text{pK}_a'$ Values

Andrews<sup>26</sup> determined the ionization constant of diphenhydramine hydrochloride at  $0^\circ$ ,  $\text{pK}_a' = 9.67$ , and  $25^\circ$ ,  $\text{pK}_a' = 9.12$  in water. These values compare well with those obtained by Lordi<sup>27</sup> of  $\text{pK}_a' = 9.00$  in water. deRoos<sup>28</sup> has determined the  $\text{pK}_a'$  at  $20^\circ$  to be 9.06 in water.

The  $pK_a'$  of Diphenhydramine Hydrochloride, USP, Lot 563463, has been determined<sup>29</sup> in a water:methanol (1:1) system to be 8.4. Since the  $pK_a'$  varies slightly with the alcohol content, the value obtained is acceptable.

### 2.13 Metal Complex Formation and Binding

Diphenhydramine hydrochloride forms complexes<sup>26</sup> with various metal ions such as  $Cu^{++}$ ,  $Co^{++}$ , and  $Ni^{++}$ .

Evidence for interaction of diphenhydramine hydrochloride with styrene-maleic<sup>30</sup> and sodium carboxymethylcellulose<sup>31</sup> has been reported.

### 3. Synthesis

The first method patented for the synthesis of diphenhydramine was by Rieveschl<sup>32</sup> in 1947, assigned to Parke, Davis & Co. The general method involves the reaction of bromodiphenylmethane with the appropriate dialkylamino alcohol in the presence of anhydrous sodium carbonate. The dialkylamino alcohol used is dimethylamino ethanol (see Figure 7). The diphenhydramine base that is formed is then converted to the HCl salt.

A variety of synthetic methods have appeared in the literature.<sup>33, 34, 35</sup> In the majority of methods, the base, diphenhydramine is formed first and then converted to the hydrochloride. In some instances, diphenhydramine hydrochloride may be formed directly by rearrangement of a quaternary ammonium salt<sup>36, 37</sup> (see Figure 8). The free base can also be formed as the result of a decarboxylation reaction<sup>38</sup> (Figure 9).

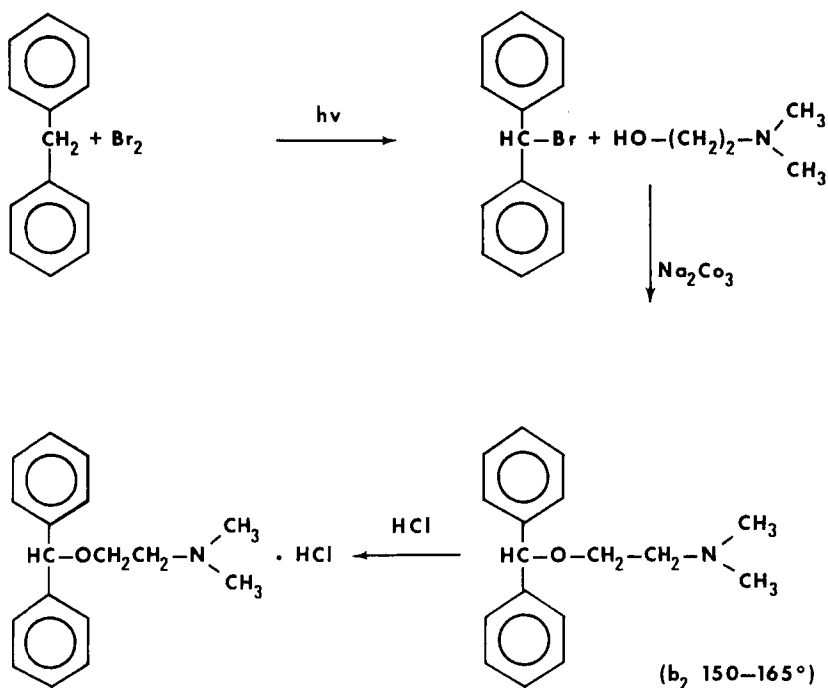
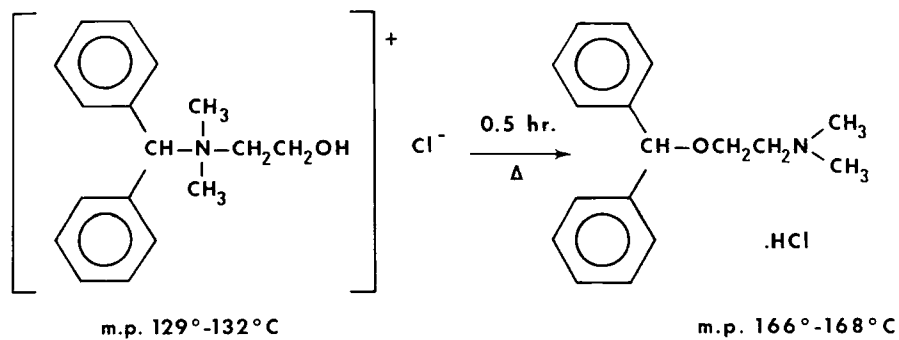
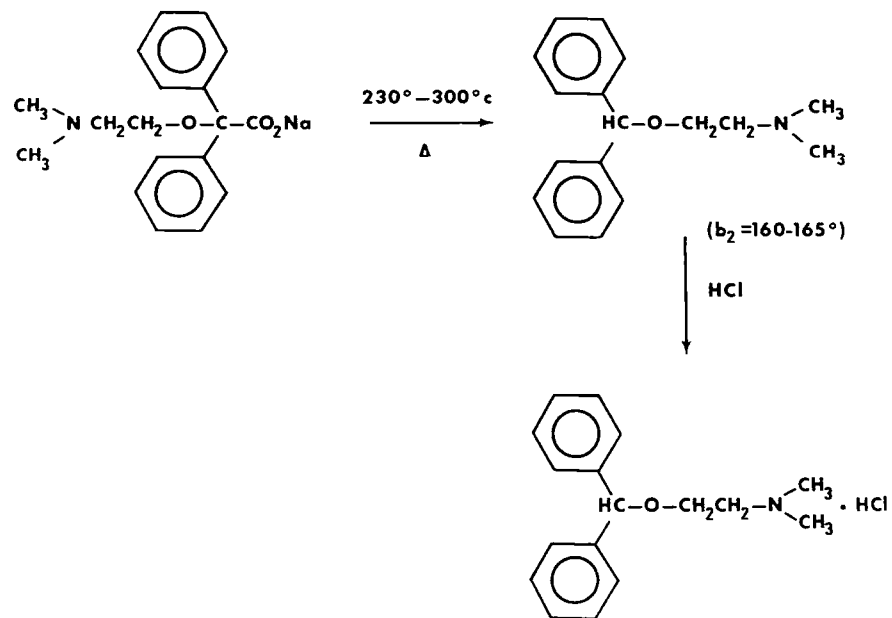


Fig. 7. Synthesis of Diphenhydramine Hydrochloride.



**Fig. 8. Synthesis of Diphenhydramine Hydrochloride: Rearrangement Reaction**



**Fig. 9. Synthesis of Diphenhydramine Hydrochloride : Decarboxylation Reaction**

#### 4. Stability - Degradation

The earliest published detailed work on the stability - decomposition of diphenhydramine hydrochloride is that of Nogami<sup>39</sup> in 1961. The kinetics of the decomposition was examined in an acidic and alkaline medium. In an acidic medium, diphenhydramine undergoes fairly rapid decomposition, whereas the compound is fairly stable in an alkaline solution. The decomposition in an acid medium is due to hydrolysis of the ether linkage. The rate determining step is first order and catalyzed by hydrogen ion. The principle degradation products are benzhydrol and 2-(dimethylamino) ethanol.

Earlier observations on the decomposition of diphenhydramine hydrochloride were in relation to the effect of hydrogen peroxide<sup>40, 41</sup> and ultraviolet light<sup>42</sup> on the compound. The decomposition products with hydrogen peroxide are toluene, benzophenone, benzyl alcohol, benzoic acid and phenolic substances in addition to dimethylaminoethanol. The benzhydrol under the conditions used undergoes further reactions. Under ultraviolet irradiation, the principle decomposition products are benzhydrol and dimethylaminoethanol.

The work by Nogami<sup>39</sup> on the stability of diphenhydramine hydrochloride was confirmed in part by deRoos<sup>43</sup> in 1963 in a study on the stability of the ether bond in a series of benzhydryl ethers.



## 5. Drug Metabolic Products - Pharmacokinetics

In the initial work by Glazko and co-workers<sup>44, 45</sup> on the metabolic fate of diphenhydramine hydrochloride, rats and guinea pigs were examined at definite times after subcutaneous injections. The highest concentrations of diphenhydramine were found in the lungs, with lower concentrations in the spleen, liver and muscles. Peak concentrations occurred in about one hour with a fairly rapid drop over a six hour period. Diphenhydramine was demonstrated in human urine in small amounts by extraction and ultraviolet absorption.

The results obtained using radioactive carbon incorporated into the  $\alpha$  position of the benzhydryl group of diphenhydramine agree with the chemical analysis.<sup>45</sup> In rats the maximum rate of excretion occurred in the first seven hours. Radioautographs prepared from urine samples showed at least six different radioactive compounds present, one of which was diphenhydramine.

Kikkawa<sup>46</sup> identified benzhydrol and dimethylaminoethanol as metabolic products in vitro and in vivo. An acidic compound was also detected, but not identified.

Drach<sup>47, 48</sup> using tritium labeled diphenhydramine in rhesus monkey plasma found the major metabolite to be a deaminated carboxylic acid derivative of diphenhydramine, (diphenylmethoxy) acetic acid. The acid, the mono- and di-dealkylated derivatives of diphenhydramine and the N-oxide derivative were identified chromatographically. The diphenyl-

methoxyacetic acid is excreted as the glutamine conjugate.

Kinkel<sup>49</sup> examined plasma levels of diphenhydramine after single dose oral administration of diphenhydramine hydrochloride capsules at different levels in human volunteers. Peak plasma levels were obtained 2 to 3 hours following the dose. In a multiple dose study, a relatively constant plasma level is obtained after three days.

Additional work<sup>50</sup> has been done on the metabolites in relation to differences observed between species. The major metabolite in all species studied, except the rat, was (diphenylmethoxy) acetic acid. This metabolite is conjugated with glutamine in the monkey and glycine in the dog.

#### 6. Identification: Microchemical Tests

The first collection of microchemical tests for detection and identification of diphenhydramine was described by Haley<sup>51</sup> in 1948. The reactions and results are summarized in Table VIII.

Molle<sup>52</sup> described an additional color reaction in 1950 in which the reagent,  $\text{H}_2\text{SO}_4$ , 90%, and  $\text{HNO}_3$ , 10%, reacts to give a red-violet color changing slowly to yellow. The resulting mixture is diluted with water to give an orange-yellow color and then the turbid mixture becomes a violet-rose. Chloroform is added and mixed well; the separated chloroform layer is violet and the aqueous layer becomes colorless. The color reaction is due to benzhydrol.

Table VIII. Identification of Diphenhydramine

Hydrochloride: Microchemical Tests<sup>51</sup>

<u>Reagent</u>	<u>Observation</u>
H <sub>2</sub> PtCl <sub>6</sub> , 5%	Definite crystalline form
Mandelin reagent	Red oily globules
Marquis reagent	Canary yellow, reddish-orange then chocolate brown
Mecke reagent	Canary yellow then reddish-orange
Froehde reagent	Canary yellow to orange-red
H <sub>2</sub> SO <sub>4</sub> , conc.	Orange
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> - H <sub>2</sub> SO <sub>4</sub>	Yellow
Resorcinol - H <sub>2</sub> SO <sub>4</sub>	Orange then reddish-orange and wine color on dilution
Furfural, 1%, over H <sub>2</sub> SO <sub>4</sub>	Orange brown and yellow-green on shaking

Table VIII(cont.)

<u>Reagent</u>	<u>Observation</u>
Chromic acid, 5%	Orange-red precipitate
Foucery reagent	Cherry-red
<u>Name Reagent Compositions:</u>	
Mandelin reagent	Ammonium vanadate, 1 g. in 100 ml. concentrated sulfuric acid
Marquis reagent	Sulfuric acid-formaldehyde. Two ml. of a 40% solution of formaldehyde mixed with 45 ml. of water and 55 ml. of concentrated sulfuric acid
Mecke reagent	Selenous acid, 0.5 g., in sulfuric acid, 100 <sup>a</sup> ml
Frohde reagent	Ammonium molybdate, 0.1% in concentrated sulfuric acid
Foucery reagent	Quinone, 1 g. in acetic acid:alcohol(5:100)

Additional microchemical tests have been described by Auterhoff<sup>53</sup>, Osol<sup>54</sup> and Neuhoﬀ<sup>55</sup>. Clarke<sup>56</sup> in 1957 gave, in addition to observations, the sensitivity of the tests used (Table IX).

Clarke<sup>57</sup> has described a method for the rapid detection of basic drugs in urine that utilizes many of the reactions described in procedures that require a minimum of equipment.

Additional reagents which form crystalline precipitates with diphenhydramine are flavianic acid<sup>58</sup>, 4,4'-dibromodibenzene-sulfonamide<sup>59</sup>, and 8-hydroxy-7-iodoquinoline-5-sulfonic acid.<sup>60</sup>

## 7. Methods of Analysis

### 7.1 Elemental Analysis

The elemental analysis of Diphenhydramine Hydrochloride, USP, Lot 563463, is presented below:

<u>Element</u>	<u>% Calculated</u>	<u>Reported</u> <sup>61</sup>
C	69.97	69.99
H	7.60	7.56
N	4.80	4.84
Cl	12.15	12.09

### 7.2 Spectrophotometric Assay

#### 7.21 Direct Methods

Methods in which the sample is diluted to the proper concentration for absorbance reading in the ultraviolet region have been reported by Setniker<sup>62</sup> and Demir<sup>63</sup>. Corrections using orthogonal functions for irrelevant

Table IX. Microchemical Identification Tests  
and Sensitivity<sup>56</sup>

<u>Reagent</u>	<u>Observations</u>	<u>Sensitivity</u>
Gold bromide/HCl	Needles, some curved	0.1 µg
Potassium triiodide	Plates	0.1 µg
Formaldehyde-sulfuric acid (Marquis)	Yellow color	0.1 µg
Ammonium vanadate	Yellow	0.1 µg
Ammonium molybdate	Yellow	0.1 µg
Selenium dioxide	Yellow	0.1 µg

absorption in tablets<sup>64</sup> has been applied with a recovery of  $99.2 \pm 1.8\%$ .

7.22 Separation Methods Prior to Spectrophotometric Assay

Column chromatography on alumina<sup>65</sup>, thin layer chromatography with an alumina layer<sup>66</sup>, direct extraction from a basic solution<sup>67</sup>, ion exchange chromatography<sup>68</sup>, and extraction from a 5% hydrochloric acid solution<sup>69, 70</sup> have been used prior to the spectrophotometric assay.

7.23 Methods Based on Conversion to Benzophenone Prior to Spectrophotometric Assay

Diphenhydramine hydrochloride is oxidized to benzophenone by either dichromate in a sulfuric acid medium<sup>71, 72</sup> or permanganate in a basic medium<sup>73</sup>. The benzophenone is either steam distilled or separated by extraction into hexane or heptane and determined spectrophotometrically.

7.24 Conversion to Chloranilic Acid Prior to Spectrophotometric Assay

Diphenhydramine has been determined in drugs by conversion to chloranil with  $\text{FeCl}_3$  in hydrochloric acid and hydrogen peroxide<sup>74</sup>. The chloranil formed is extracted and hydrolyzed to chloranilic acid (2,5-dichloro-3,6-dihydroxy-p-benzoquinone) with potassium hydroxide. The absorbance measured at 331 nm. The conversion to chloranilic acid is constant, but not 100%.

### 7.3 Colorimetric Assay

#### 7.31 Ion-Pair Extraction Methods

The method commonly used for routine control procedures is based on the extraction of diphenhydramine with methyl orange into chloroform. The procedure was initially studied by Dill and Glazko<sup>75</sup> for use in the determination of diphenhydramine in body tissues. A recent modification involves the addition of methanol after the complex is completely extracted into chloroform to prevent adsorption of the methyl orange-diphenhydramine ion-pair onto the walls of the flask<sup>76</sup>.

Other dyes that have been used for the colorimetric assay are bromocresol green<sup>77</sup>, bromocresol purple<sup>78</sup>, bromothymol blue<sup>78</sup>, eriochrome blue SE<sup>79</sup>, tetrabromophenolphthalein ethyl ester<sup>80</sup> and eosin<sup>81, 82</sup>.

#### 7.32 Ammonium Reineckate Methods

Bandelin<sup>83</sup> separated diphenhydramine as the reineckate salt followed by solution of the salt in acetone and colorimetric estimation at 525 nm. A very comprehensive paper on the identification and determination of nitrogenous bases with ammonium reineckate was presented by Kum-Tatt<sup>84</sup> in which the mole composition of diphenhydramine reineckate is given as  $C_{21}H_{28}CrN_7OS_4$ . The salt decomposes at 178 - 180°.

#### 7.33 Picric Acid Method

Picric acid has been used for the colorimetric determination of diphenhydramine in the urine of rabbits and man<sup>85</sup>.



### 7.34 Method based on Molle Reaction

Horn<sup>86</sup> examined several different procedures for the determination of diphenhydramine hydrochloride, one of which is based on the Molle reaction described earlier. The compound is reacted with a mixture of sulfuric acid and nitric acid (9:1), diluted with water and the colored compound formed extracted with chloroform. The absorbance of the chloroform layer was then determined with a filter type instrument.

### 7.35 Miscellaneous Colorimetric Methods

Diphenhydramine has been determined by extraction of a chloroform soluble complex with cobalt thiocyanate<sup>87, 88, 89</sup>. Diphenhydramine reacts in a 2:1 mole ratio and in chloroform is measured in the region 590 to 625 nm.

Diphenhydramine can be extracted from an acetate buffer, pH 5, containing iodide with a 0.5% I<sub>2</sub> solution in ethylene dichloride<sup>90</sup>.

Conditions for the complex formation of diphenhydramine with H(Tl Br<sub>4</sub>) have been examined with subsequent displacement by brilliant green<sup>91</sup>.

The color reaction with diethyl oxalate<sup>92</sup> and thiobarbituric<sup>92</sup> acid was used to determine diphenhydramine in pills<sup>93</sup>.

## 7.4 Titrimetric Analysis

### 7.41 Direct Methods of Titration

The official method<sup>1</sup> for the assay of diphenhydramine hydrochloride is by

nonaqueous titration with 0.1N perchloric acid in the presence of mercury (II) acetate using crystal violet as indicator.

Work on the nonaqueous titration method was reported by Ekablad<sup>94</sup> and acetonitrile was examined as a solvent by Mainville<sup>95</sup>. Several acid salts of diphenhydramine were titrated with perchloric acid in glacial acetic acid using the glass-glass retarded potentiometric method for detection of the endpoint<sup>96</sup>. The endpoint has been detected conductimetrically<sup>97</sup>. Diphenhydramine and acid salts can be titrated directly in anhydrous propionic acid with perchloric acid using glass-calomel electrodes<sup>98</sup>.

Diphenhydramine can also be determined using a 0.004M solution of sodium lauryl sulfate or sodium dioctyl sulfosuccinate as the titrant<sup>99, 100</sup>. The ratio of the anionic surface-active agent to the base is not integral, but approximate and constant.

#### 7.42 Separations Prior to Titration

##### 7.421 Reineckate Salt Formation

The reineckate of diphenhydramine may be decomposed by heating in an alkaline medium and a Volhard titration performed to determine the thiocyanate content<sup>101, 102</sup>. The salt formed may also be determined bromatometrically<sup>103</sup>, results are about 5% low. The chromium (III) content can be determined with very good accuracy  $\pm 0.5\%$  using a chelatometric method<sup>104</sup>.

7.422 Complexometric Method

Diphenhydramine forms an insoluble salt with bismuth which, from the reagent prepared, releases an equivalent amount of EDTA<sup>105</sup>. The liberated EDTA is titrated with 0.1M ZnSO<sub>4</sub> at pH 9.1 using eriochrome black T as indicator.

7.423 Slurry Method

Clair<sup>106</sup> and Chatten<sup>107</sup> presented slurry methods for the separation of antihistamines from tablet or capsule material. The sample is simply slurried with chloroform and filtered. The filtrate is titrated with acetous perchloric acid after glacial acetic acid is added. Tuckerman<sup>108</sup> used a mixture of magnesium oxide and siliceous earth for pre-treatment of an aqueous injection followed by washing with warm chloroform into glacial acetic acid. The base is then titrated with 0.1N perchloric acid using p-naphtholbenzein as indicator.

7.424 Ion Exchange Method

Ion exchange columns<sup>109, 110</sup> have been used in the determination of antihistamines with subsequent titration of the effluent.

7.425 Extraction Method

A collaborative study on the extraction method was reported by Heim<sup>111</sup>. The free base is extracted with ether and determined by titration. Recoveries were 99-101%.

7.43 Miscellaneous Titrimetric Methods

p-Toluenesulfonic acid in chloroform<sup>112</sup> and methanesulfonic acid in glacial

acetic acid<sup>113</sup> have been used as titrants for diphenhydramine with visual indicators. In aqueous solution, silicotungstic acid<sup>114</sup> with metanil yellow or congo red as indicator was recommended by Gramm<sup>115</sup> and the compound has also been titrated with 0.1M nitranilic acid using a polarograph for detection of the endpoint in 0.01N KCl<sup>116</sup>.

#### 7.5 Fluorometric Analysis

Weak fluorescent intensity was observed for diphenhydramine when treated with 3% H<sub>2</sub>O<sub>2</sub><sup>117</sup>, but no analytical use was made of this observation. Martin<sup>118</sup> treated a residue containing diphenhydramine with concentrated sulfuric acid and perchloric acid to obtain fluorescence at 525 nm. with excitation at 375 nm. Limit of detection observed was 0.02 µg./ml.

Glazko<sup>119</sup> used fluorescent dyes to extract diphenhydramine as an ion-pair and increased the sensitivity of direct extraction methods several hundred fold over the use of methyl orange and colorimetry.

#### 7.6 Automated Analysis

Robertson<sup>120</sup> has presented an automated method of analysis for amine drugs based on acid-dye methods. Bromocresol purple is used for diphenhydramine. The automated and manual method agree quite well with a 0.4% label claim difference. Fusari has presented an ultraviolet method for content uniformity of diphenhydramine samples<sup>121</sup>.

### 7.7 Biological Assay

Chen<sup>122</sup> used isolated guinea pig ileum for the assay of histamine and diphenhydramine in vitro. A linear relationship is observed between dose and effect.

### 7.8 Gravimetric Analysis

Uyeno<sup>123</sup> used the picrate method to determine diphenhydramine gravimetrically. The picrate is filtered, washed with water and ether, dried and weighed.

### 7.9 Chromatography

#### 7.91 Paper Chromatography

The results of chromatography on paper are summarized in Table X for diphenhydramine hydrochloride.

#### 7.92 Thin Layer Chromatography

An excellent review of the thin layer chromatography methods for diphenhydramine was presented by Comer<sup>130</sup> in 1967. Additional mobile phases used on silica gel prior to 1967 are presented by Kamp<sup>131</sup>,

(1)  $\text{CCl}_4:\text{BuOH}:\text{MeOH}:25\% \text{NH}_4\text{OH}$  (40:30:30:1), and  
(2) Petroleum ether:ether: $\text{Et}_2\text{NH}$  (20:80:1) and  
by Fuwa<sup>132</sup>,  $\text{CHCl}_3:\text{MeOH}:\text{NH}_4\text{OH}$  (98:1:1). Diphenhydramine has also been chromatographed on thin layers of alumina using

$\text{ØH}:\text{EtOH}$  (9:1) or (9:1.5)<sup>133</sup>,

$\text{ØH}:\text{EtOH}:\text{HOAc}$  (3:1.2:0.5)<sup>133</sup>;

$\text{CHCl}_3:\text{BuOH}$  (98:2)<sup>134</sup>;

$\text{CHCl}_3:\text{Me}_2\text{CO}$  (1:1)<sup>134</sup>; and

$\text{ØH}:\text{EtOH}$  (9:1)<sup>134</sup>.

Table X. Paper Chromatography of  
Diphenhydramine Hydrochloride

<u>Mobile Phase</u>	<u>R<sub>f</sub></u>	<u>Pretreatment</u>	<u>Reference</u>
n-BuOH:HOAc:H <sub>2</sub> O (40:10:50)	0.85	none	124
n-BuOH:HCl, 0.5N (90:30)	0.68	none	124
Isopropyl alc.:HCl, 0.5N (90:30)	1.00	none	124
EtOH:H <sub>2</sub> O:NH <sub>4</sub> OH (55:43:2)	0.31	Impregnated with soln. of petroleum (180-215°C) and petroleum ether	125
EtOH:H <sub>2</sub> O:NH <sub>4</sub> OH (95:3:2)	0.76	Impregnated with soln. of petroleum (180-215°C) and petroleum ether	125
n-BuOH sat. with 1N HCl	0.60	none	126
C <sub>6</sub> H <sub>6</sub> :HOAc:H <sub>2</sub> O (4:4:1)	0.70	none	126
n-BuOH sat. with pH 3 buffer	0.63	Treated with pH 3 buffer	127

Table X(cont.)

<u>Mobile Phase</u>	<u>Rf</u>	<u>Pretreatment</u>	<u>Reference</u>
n-BuOH sat. with pH 5 buffer	0.63	Treated with pH 5 buffer	127
n-BuOH sat. with pH 6.5 buffer	0.67	Treated with pH 6.5 buffer	127
n-BuOH sat. with pH 7.5 buffer	0.91	Treated with pH 7.5 buffer	127
n-BuOH:H <sub>2</sub> O (50:50) with 1 g. citric acid (use upper layer)	-	Dipped in 5% sodium dihydrogen citrate	128
n-BuOH sat. with 1N HCl	0.96	Whatman No. 4	129

Additonal systems for the thin layer chromatographic examination and detection of diphenhydramine are presented in Table XI.

The reverse phase paper chromatographic system developed by Vecerkova has been modified<sup>142</sup> to run on thin layers of silica gel in about 3 hours and will separate diphenhydramine from bromodiphenhydramine.

Thin layer chromatography has been used prior to assays by the spot-area method<sup>143</sup> and by the acid-dye reaction after elution from the plate.<sup>144</sup>

### 7.93 Gas Chromatography

#### 7.931 Direct Methods on Neutral Columns

The majority of published gas chromatographic systems for diphenhydramine hydrochloride involve injection of the free base on columns differing in polarity.

MacDonald<sup>145</sup> used a 6 ft. column of 1% SE-30 on 100-120 mesh Gas Chrom P. Retention time for diphenhydramine was 6.3 min.; column temperature 173°C.; injection port, 256°C.; argon flow rate 60 ml./min. using an argon  $\beta$ -ray ionization detector.

Kazyak<sup>146</sup> presented additional data on 1% SE-30 columns at different temperatures.

MacDonald<sup>147</sup> compared four columns in 1964 and found 0.08% PDEAS on a 120/170 glass bead column to be most successful for



Table XI. Thin Layer Chromatography  
of Diphenhydramine Hydrochloride

<u>Mobile Phase</u>	<u>Sorbent</u>	<u>Rf</u>	<u>Reference</u>
CHCl <sub>3</sub> :Me <sub>2</sub> CO (9:1)	Kieselgel G w/ fluorescent indicator	0.08	El Gendi <sup>135</sup>
MeOH	"	0.22	"
CHCl <sub>3</sub> :EtOH (9:1)	"	0.31	"
CHCl <sub>3</sub> :EtOH (8:2)	"	0.33	"
EtOAc:MeOH:NH <sub>4</sub> OH(85:10:5)	Silica Gel G	0.90	Davidow <sup>136</sup>
CHCl <sub>3</sub> :MeOH (9:1)	Silica Gel G	0.76	Bastos <sup>137</sup>
Isopropyl ether:EtOH(8:2)		0.11	"
MeOH:NH <sub>4</sub> OH(100:1.5)		0.77	"
Isopropyl ether:Me <sub>2</sub> CO(1:1)	Kieselgel G or GF	0.55 s 0.48 f	Eiden <sup>138</sup>

Table XI.(cont.)

<u>Mobile Phase</u>	<u>Sorbent</u>	<u>Rf</u>	<u>Reference</u>
Isopropanol	Kieselgel G or GF	0.50 s 0.50 f	Eiden
Benzene:dioxane:HOAc (50:40:10)	"	0.07 s	"
Cyclohexane:EtOAc:Et <sub>2</sub> NH (65:30:5)		0.51	
EtOAc:cyclohexane:dioxane: MeOH:H <sub>2</sub> O:NH <sub>4</sub> OH (50:50:10:10:1.5:0.5)	Gelman silica gel glass micro- fiber sheets	0.71	Kaistha <sup>139</sup>
EtOAc:cyclohexane: NH <sub>4</sub> OH:MeOH:H <sub>2</sub> O (70:15:2:8:0.5)	Gelman silica gel glass microfiber sheets	0.86	Kaistha <sup>139</sup>
EtOAc:cyclohexane:MeOH: NH <sub>4</sub> OH (70:15:10:5)	"	0.91	"

Table XI. (cont.)

<u>Mobile phase</u>	<u>Sorbent</u>	<u>Rf</u>	<u>Reference</u>
EtOH:HOAc:H <sub>2</sub> O (50:30:20)	Silica gel G	N.A.	Boonen <sup>140</sup>
EtOH:NH <sub>4</sub> OH (28%) (98:2)	"	0.55	Quan <sup>141</sup>

N. A. = not available

s = plates prepared in the laboratory

f = purchased plates

the antihistamines. Diphenhydramine has a retention time of 2.5 min. on a 6' column at 175°C. with an argon flow rate of 60 ml./min.

Jain<sup>148</sup> used 1% Hi-Eff-8B on 100/120 mesh silanized Gas Chrom P. Retention times of diphenhydramine given are relative to methapyrilene; 0.43 at 160°C. and 0.46 at 190°. Gas chromatography was used for the determination of diphenhydramine in blood after an extraction with acetone-ether.

A mixture of Hi-Eff-8BP, 1%, and 10% SE-52 on Gas Chrom Q was used by Rader<sup>149</sup> in application to single and multiple component drugs. The column temperature was 220°C. Relative retention time was 0.83 to pentobarbital.

Patel<sup>150</sup> presented a general article on gas chromatography in which diphenhydramine was chromatographed on 3% Phenyl Methyl Silicone (OV 17) on Gas Chrom Q at 175° (6 ft., 4 mm. I.D.).

A rapid, direct analysis of antihistamines was reported by Reiss<sup>151</sup> in which the sample is dispersed in water, diluted to volume, filtered and injected. Relative retention times to chlorpheniramine maleate are reported on two columns both 4 ft. x 0.25 in. o.d. glass: 2% SE-30 and 2% Carbowax 20M on 80/100 mesh Diatoport S, 0.52; and 10% silicone oil DC-200 on 60/80 mesh Diatoport S, 0.63. Column temperature was 210°C.

7.932 Direct Methods on Basic Columns

Steele reported<sup>152</sup> on the use of a column with 5% Apiezon L and 4.5% potassium hydroxide. Column temperature was 138° for the first 6 min. after injection and then raised at 6°C./min. to 275°C. Retention time for diphenhydramine was 3.64 min.

7.933 Oxidation to Benzophenone Prior to Gas Chromatography  
Oxidation with 0.033M

Cr<sub>2</sub>O<sub>3</sub> for 60 min. converts diphenhydramine to benzophenone which can be determined in nanogram amounts with a precision of 1.5% using electron capture gas chromatography<sup>153</sup>.

7.94 Column Chromatography

Levine<sup>154</sup> reported on the partitioning of diphenhydramine between 2N HCl and chloroform on a Celite column. The diphenhydramine is eluted with a mixture of 90 ml. chloroform containing 1 ml. of acetic acid after a prewash of the column with diethyl ether.

Doyle<sup>19</sup> has examined distribution diagrams and selected Celite partition chromatographic systems for various separations on the basis of the diagrams. The effects of solvent composition on the column partition chromatography of amines has also been examined by Doyle<sup>155</sup> and some information on diphenhydramine was presented.

7.95 Electrophoresis

The electrophoresis of diphenhydramine has been carried out by Baruffini<sup>124</sup>

in different pH buffers at 7 volts/cm. for 3 hours. Migration is optimal at low pH with 82 mm. displacement toward the cathode at pH 2.1.

Derlikowski<sup>156</sup> examined the electrophoresis of diphenhydramine in 1965. Zones were detected with a Dragendorff reagent.

The literature has been reviewed through 1972.

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**ECHOTHIOPHATE IODIDE**

*Raymond D. Daley*

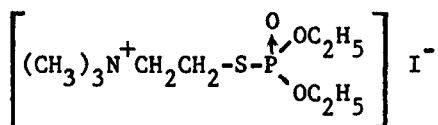
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## 1. Description

### 1.1 Name, Formula, Molecular Weight

The Chemical Abstracts name for echothiophate iodide is 2-[(diethoxyphosphinyl)thio]-N,N,N-trimethyl ethanaminium iodide, starting with Volume 76 (1). Previously the Chemical Abstracts name was (2-mercapto-ethyl)trimethylammonium iodide S-ester with 0,0-diethyl phosphorothioate. The CAS Registry No. is [513-10-0]. The British Pharmaceutical Codex lists the compound as ecothiophate iodide (2), and The Merck Index lists several other names (3).



$\text{C}_9\text{H}_{23}\text{INO}_3\text{PS}$

Mol. Wt.: 383.23

### 1.2 Appearance, Color, Odor

White crystalline powder, with a slight mercaptan-like odor.

## 2. Physical Properties

### 2.1 Infrared Spectra

Figure 1 is an infrared spectrum of one of the crystalline forms of echothiophate iodide. This form will be designated as Form I in Section 2.7, Crystal Properties. The spectrum was run in several sections: (a) from 200 to 540  $\text{cm}^{-1}$  as a mineral oil mull on polyethylene; (b) from 470 to 1360  $\text{cm}^{-1}$  as a mineral oil mull between potassium bromide plates, with the 900 to 1055  $\text{cm}^{-1}$  and 1220 to 1275  $\text{cm}^{-1}$  regions run in two thicknesses; (c) from 1360 to 4000  $\text{cm}^{-1}$  as a perfluorinated oil mull between potassium bromide plates. The spectrum was obtained with a Beckman IR-12 spectrophotometer.

Some of the absorption bands can be assigned as follows (4):

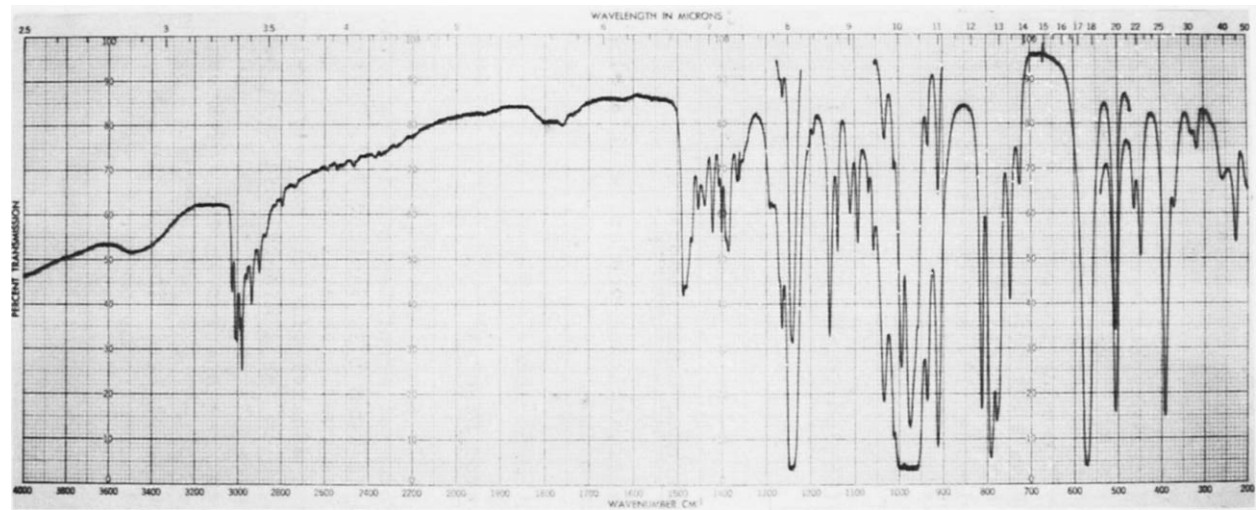


Figure 1. Infrared spectrum of echothiophate iodide, perfluorinated and mineral oil mull.

3000 $\text{cm}^{-1}$	C-H Stretching
1240 $\text{cm}^{-1}$	P=O Stretching
1157 $\text{cm}^{-1}$	P-O-Ethyl Vibration
975 $\text{cm}^{-1}$	P-O-C (Alkyl) Vibration

## 2.2 Nuclear Magnetic Resonance Spectra

Two proton magnetic resonance spectra of echothiophate iodide are shown in Figures 2 and 3. These were run in  $\text{D}_2\text{O}$  and in  $\text{CDCl}_3$ , on a Varian A-60A 60 MHz NMR spectrometer, with a tetramethylsilane reference (5).

The NMR spectra of organic phosphorus compounds are complicated by coupling of the proton signals with that of phosphorus. This coupling causes readily observable splitting of the lines from methylene protons in the groups  $\text{P-O-CH}_2$  and  $\text{P-S-CH}_2$ , with  $J_{\text{PH}}$  coupling constants of 9 Hz (6).

The proton NMR spectral assignments are given in Table I (5).

A phosphorus NMR scan indicates a chemical shift of about -28 ppm for the phosphorus in echothiophate iodide in aqueous solution, relative to a phosphoric acid reference (7). This is consistent with literature values for this structure (8).

## 2.3 Ultraviolet Spectra

Figure 4 shows the ultraviolet absorption spectrum of echothiophate iodide, run on a Cary Model 14 spectrophotometer. The sample was dissolved in water. The maximum at 226 nm has an absorptivity of  $1.34 \times 10^4$  liters per mole cm. This absorption is essentially that of the iodide ion (the ultraviolet spectrum of a potassium iodide solution exhibits a maximum at 226 nm with an absorptivity of about  $1.35 \times 10^4$  liters per mole cm).

## 2.4 Mass Spectra

Attempts to obtain the mass spectrum of echothiophate iodide were unsuccessful; the compound apparently

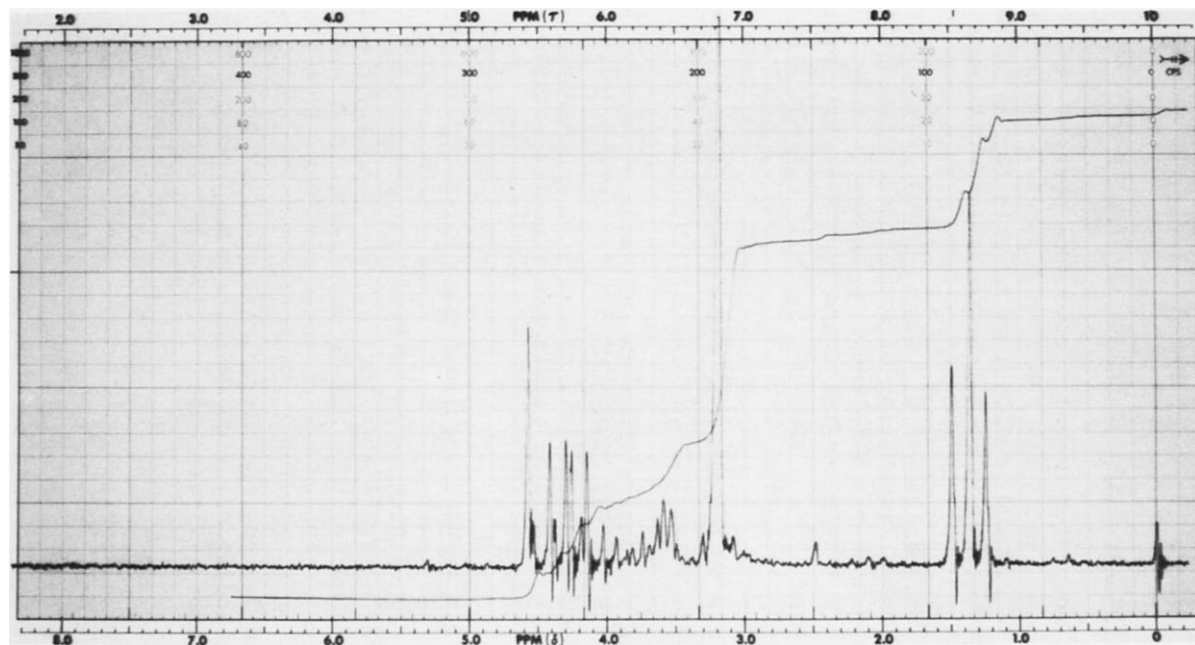


Figure 2. Proton NMR spectrum of echothiophate iodide,  $D_2O$  solution.

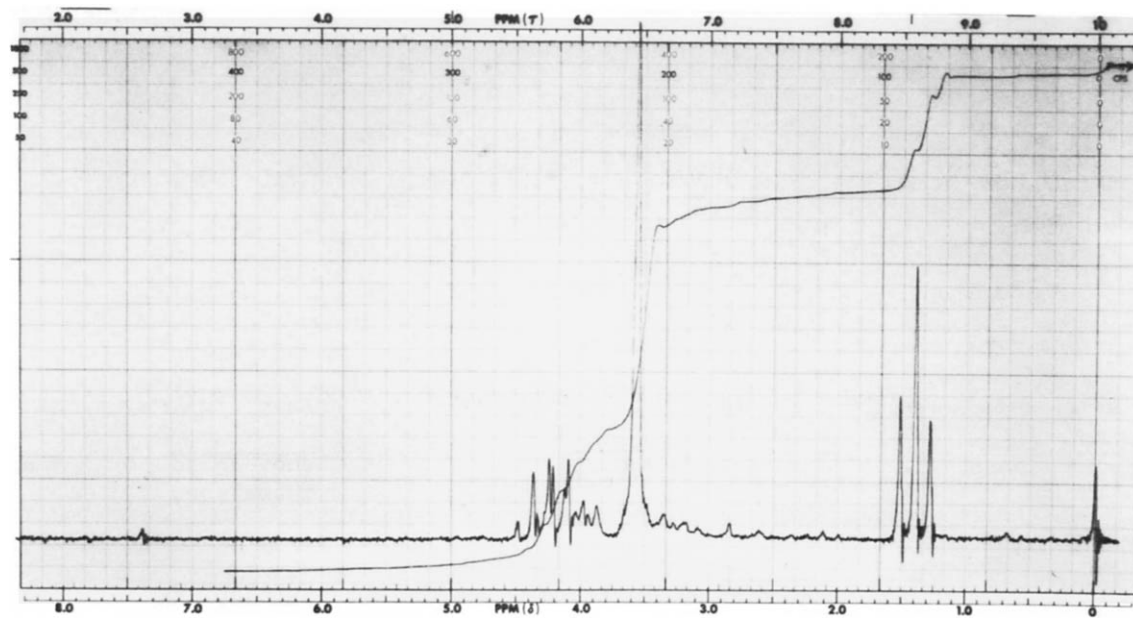


Figure 3. Proton NMR spectrum of echothiophate iodide,  $\text{CDCl}_3$  solution.



TABLE I  
NMR SPECTRAL ASSIGNMENTS FOR ECHOTHIOPHATE IODIDE (5)

<u>Number of Protons</u>	<u>Assignment of Protons</u>	<u>CDCl<sub>3</sub></u>	<u>D<sub>2</sub>O</u>
6	a, CH <sub>3</sub> (Ethyl)	1.40, t, J <sub>a,e</sub> 7.0 Hz	1.36, t, J <sub>a,e</sub> 7.25 Hz
2	b, C-CH <sub>2</sub> -S	3.3, m	3.2, m
9	c, CH <sub>3</sub> -N <sup>+</sup>	3.55, s	3.16, s
2	d, -CH <sub>2</sub> -N <sup>+</sup>	3.9, m	3.55, m
4	e, -CH <sub>2</sub> -O-P	4.22, m, J <sub>e,a</sub> 7.0 Hz	4.28, m, J <sub>e,a</sub> 7.25 Hz

t = triplet

m = multiplet

s = singlet

J = coupling constant

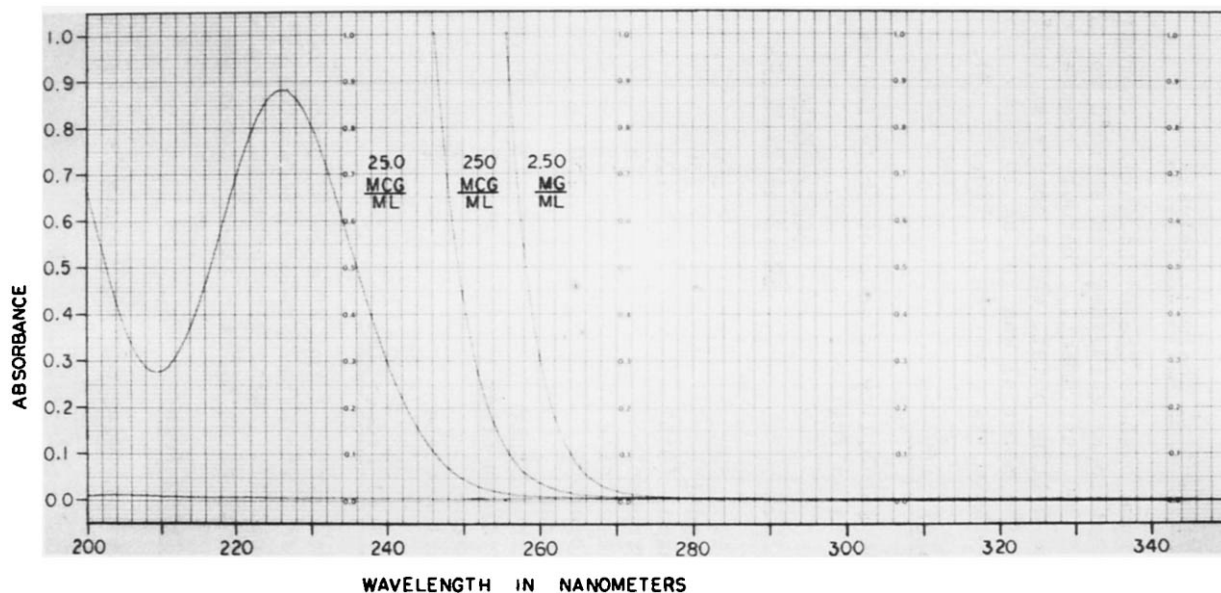


Figure 4. Ultraviolet spectrum of echthiophate iodide in aqueous solution vs water, 1 cm cells; 25.0 mcg/ml, 250 mcg/ml, 2.50 mg/ml.

decomposed in the heated inlet of the mass spectrometer (5).

## 2.5 Differential Thermal Analysis

Figure 5 shows the differential thermal analysis curve of echothiophate iodide, run at 10°C per minute on a Dupont Model 900 instrument. The only thermal event below 200°C is the melting point, which occurs at 122°C on this scan. When the material was run at 20°C per minute, the melting endotherm was observed at 125.5°C (9).

## 2.6 Solubility

The solubility of echothiophate iodide at room temperature is as follows:

<u>Solvent</u>	<u>Approximate Solubility, mg/ml</u>
Water	> 500
Methanol	> 250
Ethanol (95%)	> 120
2-Propanol	4
Acetonitrile	25
Chloroform	250
Acetone	8
Diethyl Ether	< 1
Petroleum Ether	< 1
Benzene	< 1
Ethyl Acetate	< 1

## 2.7 Crystal Properties

Two crystal forms of echothiophate iodide have been observed. The x-ray powder diffraction patterns are given in Table II. These were obtained with a Norelco diffractometer, using nickel-filtered copper K $\alpha$  radiation.

## 2.8 Melting Point

The following melting points have been reported:

124-4.5°C	(10)
138	(11)

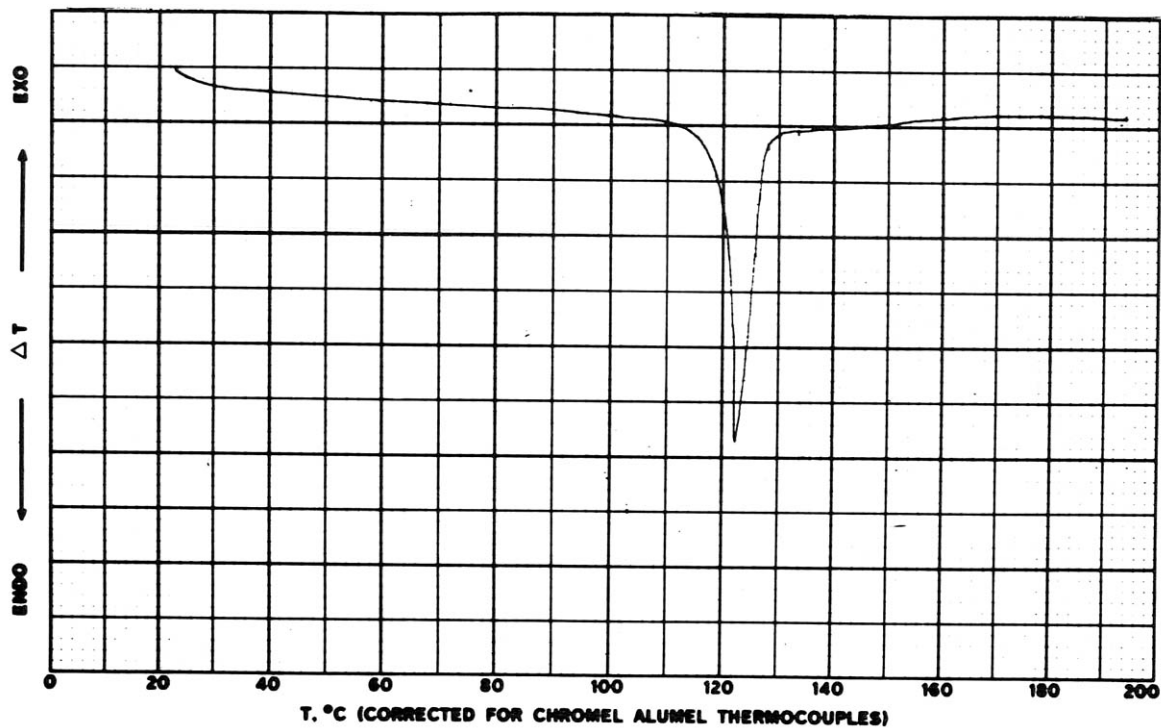


Figure 5. Differential thermal analysis scan of echothiophate iodide.

TABLE IIX-Ray Powder Diffraction Data  
for Echothiophate Iodide

<u>Form I</u>		<u>Form II</u>	
<u>d, A°</u>	<u>I/I<sub>1</sub></u>	<u>d, A°</u>	<u>I/I<sub>1</sub></u>
10.54	23	10.00	64
8.00	9	6.79	24
6.14	99	6.68	15
5.90	66	5.40	100
5.49	22	4.99	40
5.30	41	4.64	47
5.01	10	4.45	56
4.86	100	4.37	56
4.75	52	4.21	22
4.49	10	4.06	28
4.33	25	4.00	17
4.21	56	3.95	92
4.16	66	3.81	24
4.10	8	3.73	20
4.01	46	3.60	30
3.94	13	3.41	35
3.82	60	3.34	30
3.69	21	3.28	4
3.61	2	3.22	24
3.54	70	3.14	13
3.49	92	3.07	12
3.45	22	2.96	24
3.40	33	2.92	13
3.28	11	2.86	7
3.24	6	2.81	24
3.20	22	2.77	6
3.16	4	2.71	21
3.12	15	2.67	7
3.06	9	2.43	13
3.01	3	2.34	11
2.97	8		
2.95	13		
2.92	15		
2.89	12		
2.82	31		

TABLE II (Cont'd.)

<u>d, A°</u>	<u>I/I<sub>1</sub></u>	<u>d, A°</u>	<u>I/I<sub>1</sub></u>
2.74	10		
2.70	16		
2.67	25		
2.58	13		
2.53	4		
2.50	6		
2.48	4		
2.44	14		
2.40	3		
2.37	4		
2.31	4		
2.27	7		

### 3. Synthesis

Two methods for preparing echothiophate iodide have been published. The reactions are shown in Figure 6.

In the first method (10), the sodium salt of dimethylaminoethyl mercaptan is prepared by treating dimethylaminoethyl mercaptan hydrochloride with sodium. The product is treated with diethylchlorophosphate to yield 0,0-diethyl-S- $\beta$ -dimethylaminoethyl thiophosphate. This material is treated with methyl iodide to make echothiophate iodide.

In the second method (11), a mixture of diethylchlorophosphate, dimethylaminoethyl mercaptan, and triethylamine in ether is refluxed. The mixture is filtered to remove the insoluble triethylammonium chloride and distilled to obtain the 0,0-diethyl-S- $\beta$ -dimethylaminoethyl thiophosphate. This material is treated with methyl iodide to make echothiophate iodide.

### 4. Stability -- Degradation

Hussain et al (12) have shown that echothiophate iodide decomposes by at least two mechanisms. In the pH range 2.4 to 5, the major reaction is hydrolysis of one of the C-O bonds to form ethanol and the monoethyl ester.

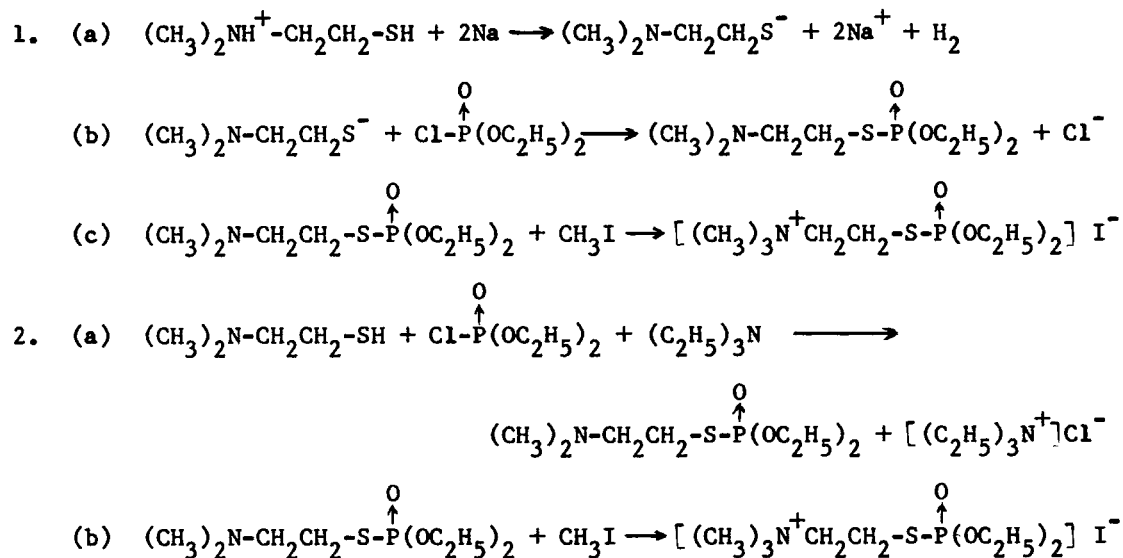


Figure 6. Synthetic Methods for Echothiophate Iodide.

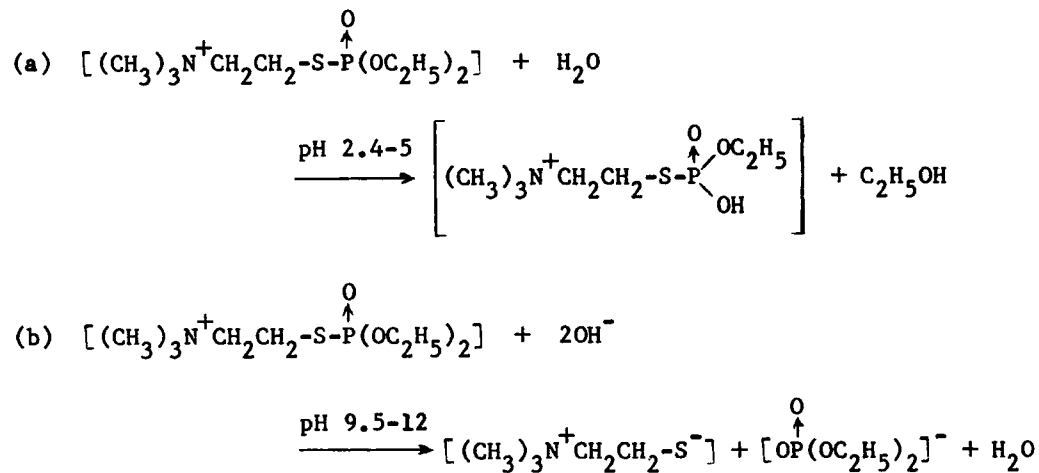


Figure 7. Degradation of Echothiophate Iodide.



In the pH range 9.5 to 12, the major reaction is hydrolysis of the S-P bond to yield (2-mercaptoethyl) trimethylammonium iodide and diethylphosphoric acid. These reactions are shown in Figure 7. At intermediate pH, both reactions occur.

## 5. Drug Metabolic Products

No metabolic products have been reported.

## 6. Methods of Analysis

### 6.1 Elemental Analysis

The elemental composition of echothiophate iodide is as follows:

<u>Element</u>	<u>% Theory</u>
Carbon	28.21
Hydrogen	6.05
Iodine	33.11
Nitrogen	3.65
Oxygen	12.52
Phosphorus	8.08
Sulfur	8.37

### 6.2 Ultraviolet Spectrophotometric Analysis

#### 6.21 Direct Ultraviolet Absorption Measurement

Although the ultraviolet absorption at 226 nm has been used in hydrolysis studies (12), it was useful only because it increased as the echothiophate iodide hydrolyzed. The iodide ion is the principal absorbing species at this wavelength in echothiophate iodide solutions (see Section 2.3), so that this maximum can be used only indirectly to measure the echothiophate cation concentration.

#### 6.22 Indirect Ultraviolet Absorption Method

An ultraviolet assay for echothiophate cation is possible, using hydrolysis to thiocholine, followed by reaction with 4,4'-dithiopyridine to form an

ultraviolet absorbing material (13). The method is based on that of Grasseti and Murray (14). The ultraviolet quantitation is essentially an alternative to the titration described in Section 6.3, but requires less sample.

The echothiophate iodide is hydrolyzed quantitatively to thiocholine in 20 minutes in pH 12.0 phosphate buffer. The hydrolyzed sample is then treated with a solution of 4,4'-dithiopyridine in pH 2.3 phosphate buffer. The final solution has a pH of 6.2, and the 4,4'-dithiopyridine reacts with thiocholine to form 4-thiopyridone. 4-Thiopyridone has an absorption maximum at 323 nm. A blank is prepared by mixing a portion of the original echothiophate iodide solution, before hydrolysis, with a solution of 4,4'-dithiopyridine. Echothiophate iodide assayed by the titration procedure is used as a standard (13).

### 6.3 Titrimetric Assay Method

The USP method for assay of raw material and dosage forms is iodimetric titration of the thiocholine formed by hydrolysis of the echothiophate iodide. In the USP XVIII procedure, the sample is hydrolyzed with sodium hydroxide (15). It has been shown recently that greater specificity is obtained when hydrolysis is conducted with a pH 12 buffer (16). It is necessary for the pH to be as high as 12 in order that the hydrolysis be completed in 20 minutes. On the other hand, too high a pH increases interference from possible impurities (16).

### 6.4 Thin Layer Chromatography

The following systems have been found useful for separating echothiophate iodide from possible degradation products: (a) Silica Gel G (E. Merck) with methanol-water-concentrated ammonium hydroxide (2:2:1) developing solvent and iodine vapor detection (17); (b) Cellulose F (E. Merck) with butanol-acetic acid-water (4:1:5) developing solvent and iodine vapor detection (18).

### 6.5 Other Tests

A microscopic identity test for echothiophate iodide has been reported. Echothiophate iodide in aqueous solution forms a crystalline precipitate with ammonium reineckate (19).

### 7. Acknowledgments

The writer wishes to thank Dr. B. T. Kho for his review of the manuscript, Dr. G. Schilling of Ayerst Research Laboratories and Dr. W. E. Krueger of the State University of New York at Plattsburgh for their NMR data and interpretation, the library staff for their literature search, the numerous other contributors who provided information for this profile, and Mrs. Kay Mannan for typing the profile.

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The above references cover the literature through 1972.

**ETHYNODIOL DIACETATE**

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Contents

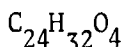
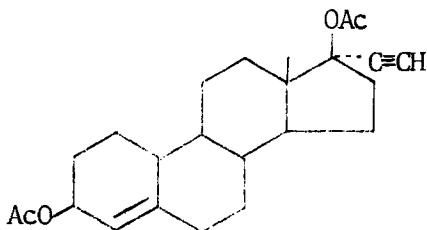
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    - 6.61 Column Chromatography
    - 6.62 High Pressure Liquid Chromatography
    - 6.63 Thin Layer Chromatography
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8. References

## ETHYNODIOL DIACETATE

### 1. Description

#### 1.1 Name, Formula, Molecular Weight

Ethynodiol Diacetate is 19-Nor-17 $\alpha$ -pregn-4-en-20-yne-3 $\beta$ , 17-diol Diacetate.



Molecular Weight: 348.52

#### 1.2 Appearance, Color, Odor

Ethynodiol diacetate is a white to off-white, essentially odorless powder.

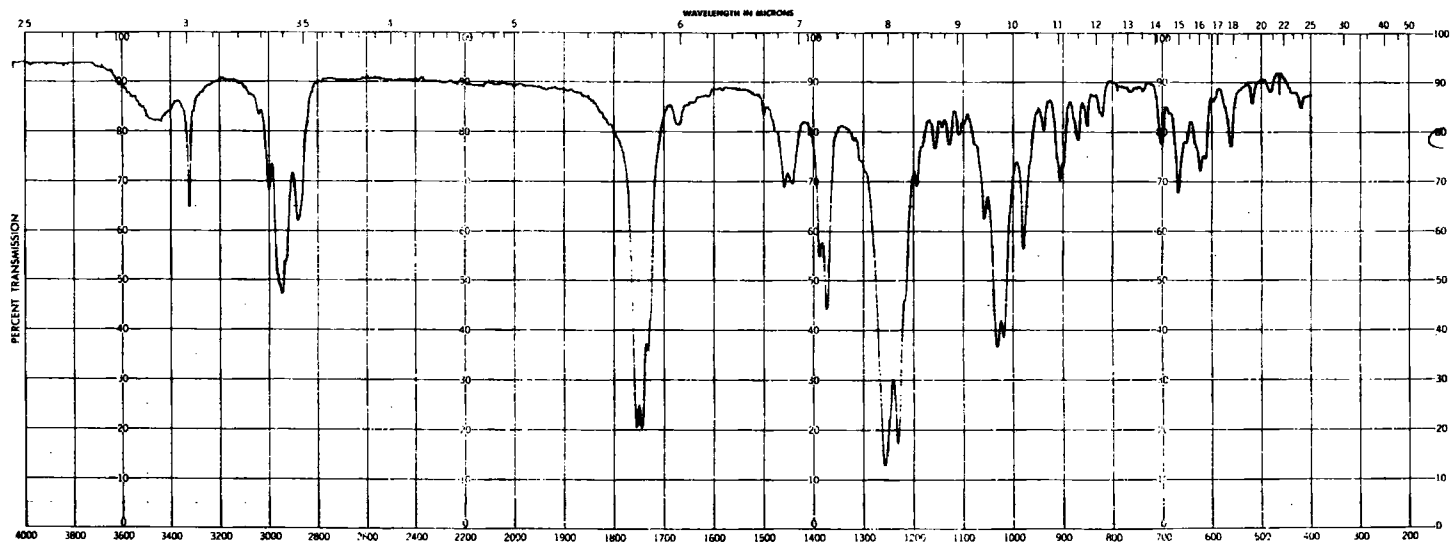
### 2. Physical Properties

#### 2.1 Infrared Spectrum

The infrared absorption spectrum of an ethynodiol diacetate reference standard compressed in a KBr pellet is shown in Figure 1. The compound exhibits essentially the same infrared spectrum in chloroform solution. The following assignments have been made for absorption bands in Figure 1 .<sup>1</sup>

<u>cm. <sup>-1</sup></u>	<u>Assignment</u>
3315	C $\equiv$ CH : Acetylenic C-H stretching
1740	C=O : Acetate Carbonyl stretching
1670	C=C : Ethylenic stretching

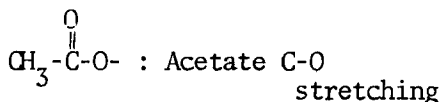
FIG. 1: INFRARED SPECTRUM OF ETHYNODIOL DIACETATE





# ETHYNODIOL DIACETATE

1275, 1028



## 2.2 Nuclear Magnetic Resonance Spectrum

The NMR spectrum of ethynodiol diacetate in deuterated chloroform is shown in Figure 2. Spectral assignments are as follows:<sup>2</sup>

<u>Chemical Shift</u> <u>(ppm)</u>	<u>Type</u>	<u>Assignment</u>
5.00-5.41	Broad Singlet	Protons at C-3 and C-4
2.58	Singlet	C≡CH : Ethynyl proton
2.03	Singlet	$-\text{O}-\overset{\text{O}}{\underset{\parallel}{\text{C}}}-\text{CH}_3$ : Acetyl methyl protons
0.90	Singlet	$-\text{CH}_3$ : C-18 methyl protons

## 2.3 Ultraviolet Spectrum

Ethynodiol diacetate does not absorb between 420 nm and 210 nm. A peak is observed at 204 nm which is not convenient for quantitative determination.

The USP XVIII assay procedure involves acid hydrolysis of the compound in methanolic 0.7 N HCl, for 10 minutes on a steam bath. The resulting solution of diene exhibits the absorption spectrum shown in Figure 3, with maxima at about 229 nm, 236 nm and 244 nm. The peak at 236 nm is used for quantitative determination.<sup>3</sup>

## 2.4 Mass Spectrum

The low resolution mass spectrum of ethynodiol diacetate shown in Figure 4 was obtained with an AEI Model MS-30

FIG. 2: NUCLEAR MAGNETIC RESONANCE SPECTRUM  
OF ETHYNODIOL DIACETATE

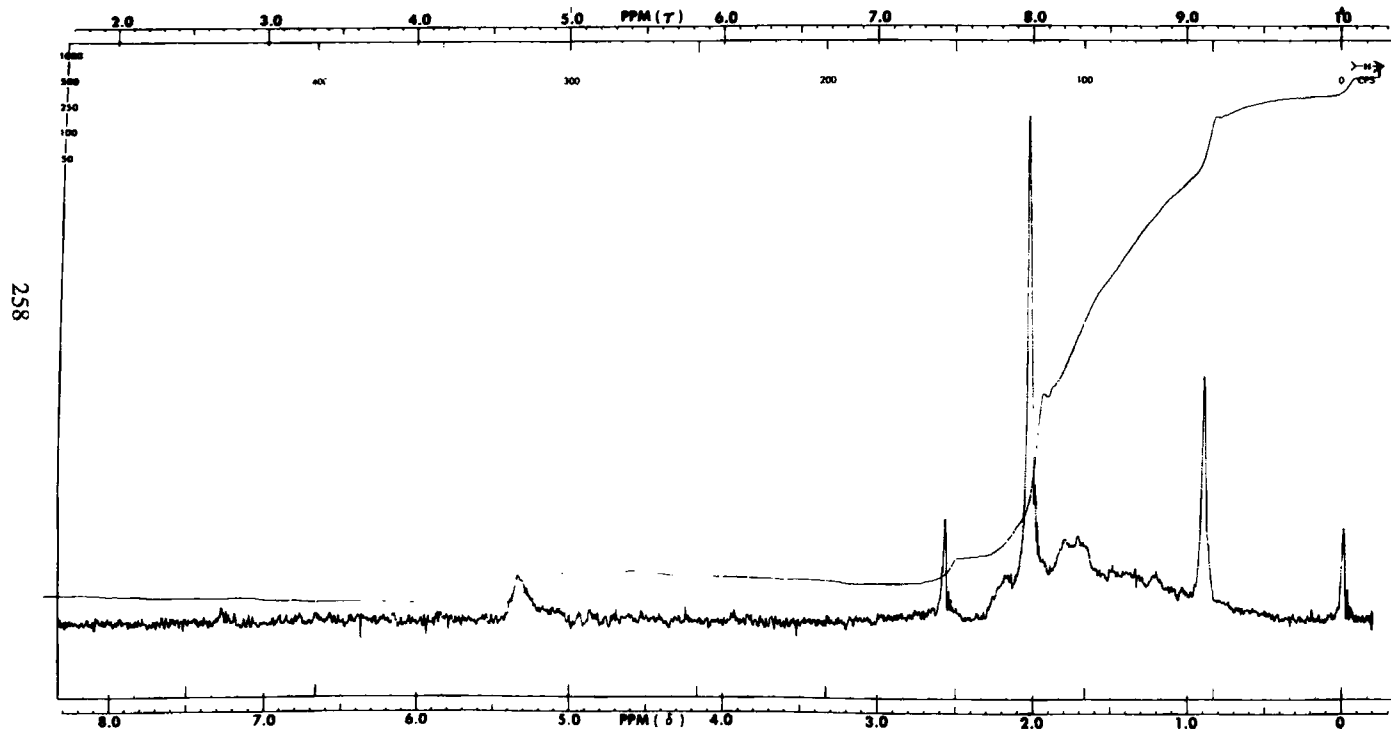


FIG. 3: ULTRAVIOLET SPECTRUM OF ETHYNODIOL DIACETATE

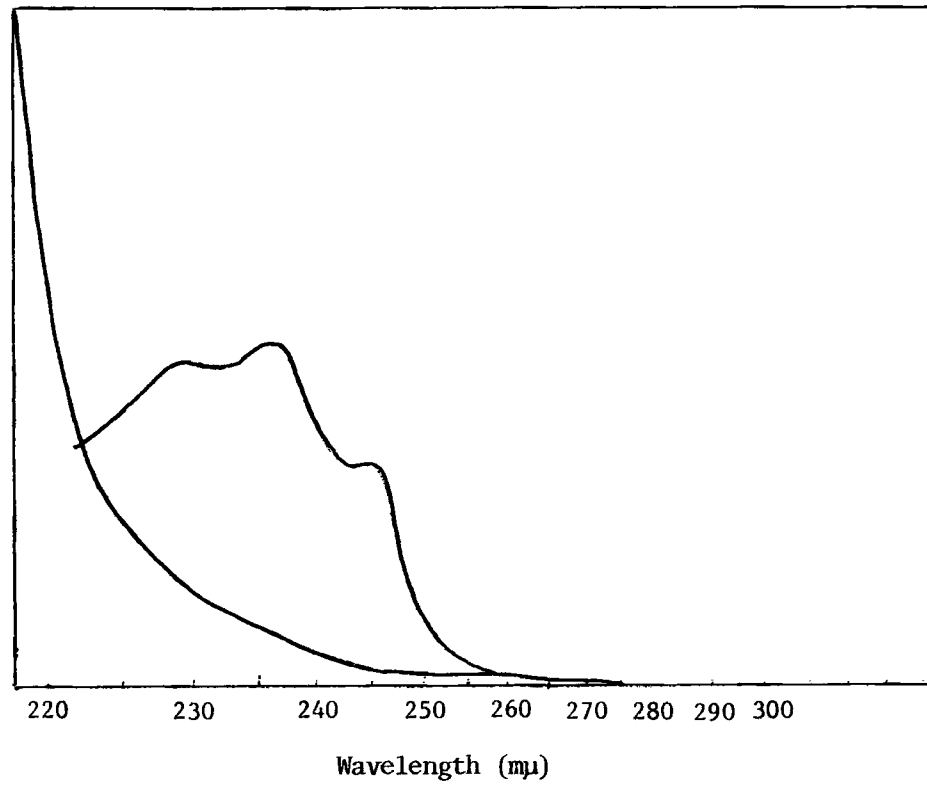
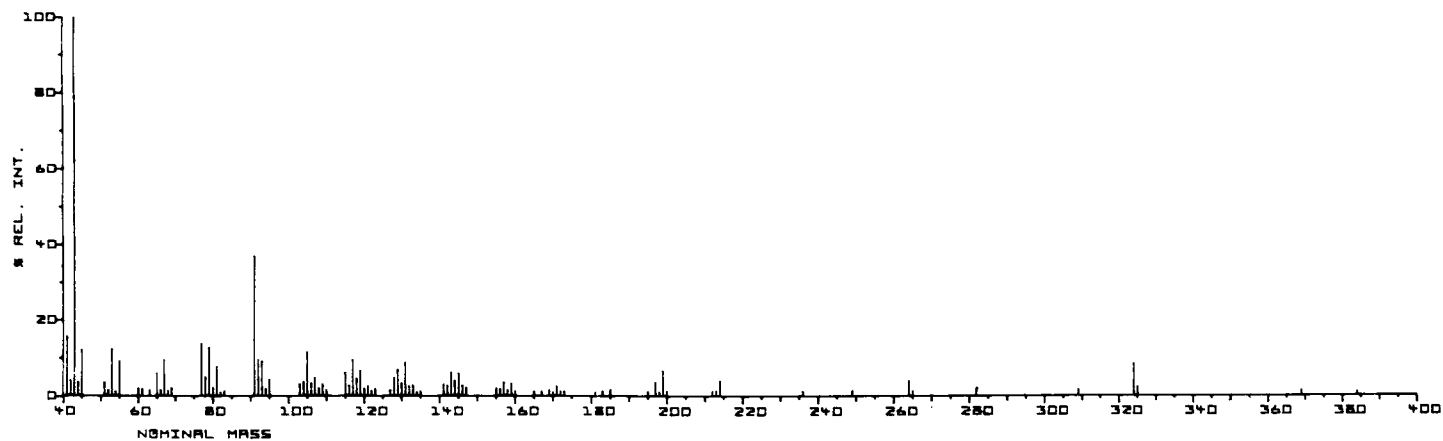


FIG. 4: MASS SPECTRUM OF ETHYNODIOL DIACETATE



# ETHYNODIOL DIACETATE

mass spectrometer. A molecular ion was observed at m/e 384. The base peak in the spectrum was at m/e 43, corresponding to  $\text{CH}_3\text{CO}^+$ . Structure assignments are summarized below:<sup>4 5</sup>

<u>m/e</u>	<u>Assignment</u>	<u>% Relative Intensity</u>
384	$\text{M}^+$	1.0
369	$\text{M} - \text{CH}_3 \cdot$	1.4
342	$\text{M} - \text{CH}_2\text{CO}$	0.2
324	$\text{M} - \text{CH}_3\text{COOH}$	8.5
309	$\text{M} - (\text{CH}_3 \cdot + \text{CH}_3\text{COOH})$	1.6
282	$\text{M} - (\text{CH}_2\text{CO} + \text{CH}_3\text{COOH})$	2.2
264	$\text{M} - (2 \text{CH}_3\text{COOH})$	4.0
249	$\text{M} - (2 \text{CH}_3\text{COOH} + \text{CH}_3 \cdot)$	1.4
43	$\text{CH}_3\text{CO}^+$	100.0

## 2.5 Optical Rotation

The following specific rotation values in chloroform have been reported.<sup>5</sup>

$$[\alpha]_{365}^{27} = -282.0^\circ$$

$$[\alpha]_{436}^{27} = -164.0^\circ$$

$$[\alpha]_{546}^{27} = -91.0^\circ$$

$$[\alpha]_{578}^{27} = -78.7^\circ$$

$$[\alpha]_{589}^{27} = -75.0^\circ$$

## 2.6 Melting Range

The melting range given in the USP XVIII is 126° to 132°C.<sup>6</sup>

## 2.7 Differential Scanning Calorimetry

The DSC thermogram of ethynodiol diacetate obtained at a heating rate of 5°C/minute is shown in Figure 5. The endothermic change observed at about 126°C corresponds to the melting of the drug. The decomposition temperature is 228°C.<sup>7</sup>

## 2.8 Thermogravimetric Analysis

The TGA spectrum of ethynodiol diacetate in Figure 6 was produced under a nitrogen sweep at a heating rate of 10°C/minute. A rapid weight loss was observed from about 210°C to 260°C. Another rapid weight loss was seen starting at about 400°C.<sup>8</sup>

## 2.9 Solubility

Solubilities in various solvents at 25°C are given in the following table:<sup>7</sup>

<u>Solvent</u>	<u>Solubility, mg./ml.</u>
Water	0.0014
Methanol	>50
Ethanol	>50
Chloroform	>50
Heptane	18

ETHYNODIOL DIACETATE

FIG. 5:  
DSC SPECTRUM

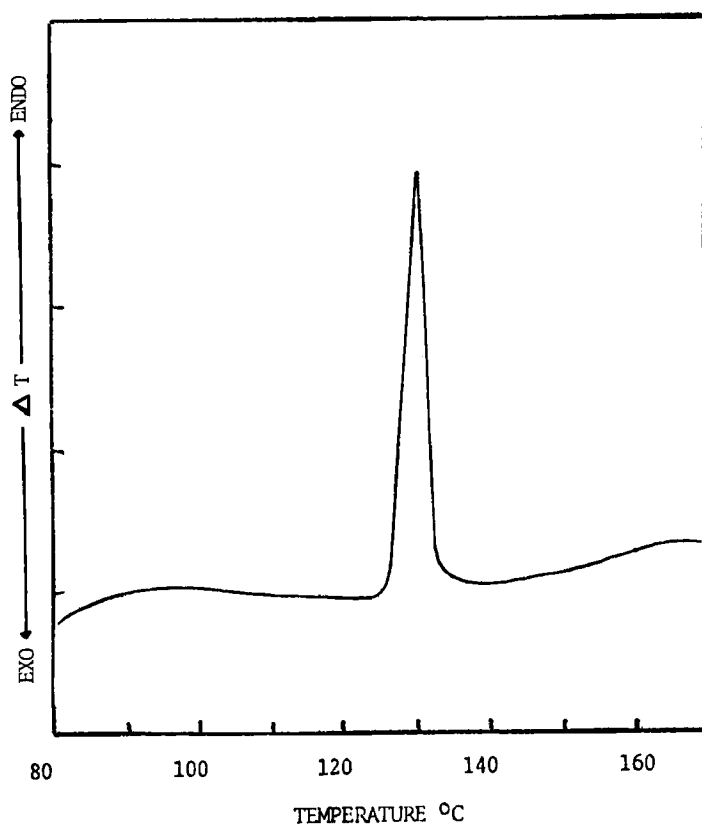
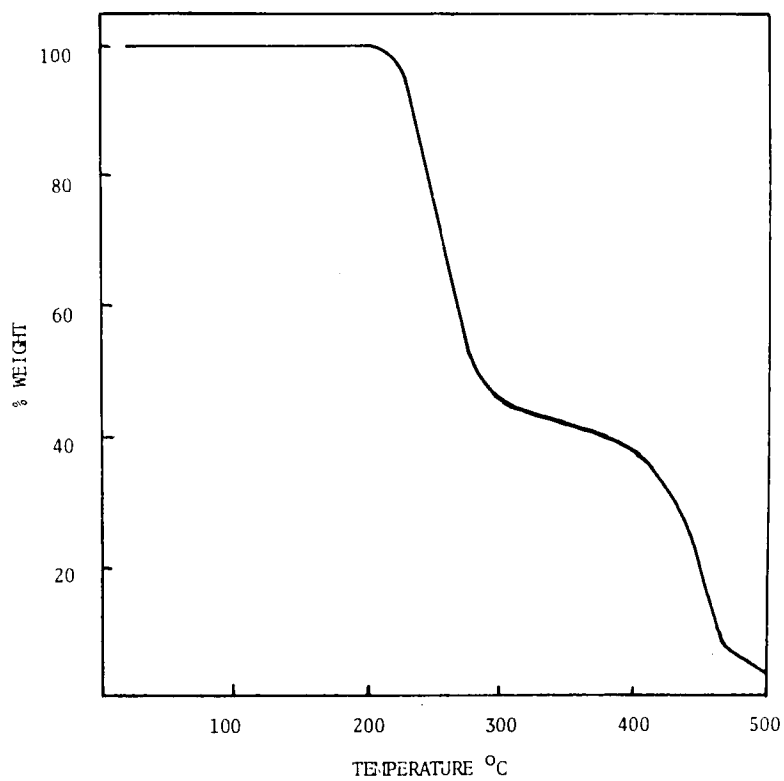


FIG. 6:  
TGA SPECTRUM OF ETIYNODIOL DIACETATE





### 3. Synthesis

Ethynodiol diacetate has been synthesized by routes utilizing both estradiol 3-methyl ether (I) and 3 $\beta$ -hydroxyandrost-5-en-17-one (II) as starting materials.

In the former method, <sup>9, 10, 11</sup> outlined in Figure 7, estradiol 3-methyl ether (I) is reduced by the Wills-Nelson modification of the Birch procedure<sup>12</sup>, to give the 1,4-dihydro derivative (III). Oppenauer oxidation of (III)<sup>13</sup> yields the 17-ketone (IV), which is then ethynylated,<sup>14</sup> giving the enol ether intermediate, (V). Reaction of (V) with dilute acetic acid produces norethynodrel<sup>15</sup> (VI). Treatment of either (V) or (VI) with aqueous mineral acid gives norethindrone (VII), which is then converted to ethynodiol (VIII) by reduction with sodium borohydride<sup>10, 16</sup>. The diol is then diacetylated with acetic anhydride and pyridine, yielding ethynodiol diacetate (IX).<sup>11</sup>

Alternatively, as shown in Figure 8, peracid treatment of 3 $\beta$ -hydroxyandrost-5-en-17-one (II) yields the 5,6  $\alpha$ -epoxide (X).<sup>17</sup> Perchloric acid cleavage of (X) results in the 5,6-diol (XI); acetylation then gives the 3, 5,6-triacetate (XII), which reacts selectively with bicarbonate to give the 3 $\beta$ ,6 $\beta$ -diol-5 $\alpha$ -acetate (XIII). Selective acetylation at C-3 followed by lead tetraacetate and iodine functionalization of C-19 then yields the 6 $\beta$ , 19-oxide (XIV). Bicarbonate hydrolysis of (XIV) followed by chromic acid oxidation of the resulting alcohol affords the key intermediate (XV), which, when treated with zinc and zinc chloride in methanol gives 19-hydroxyandrostenedione (XVI). Treatment of (XVI) with chromic acid affords the acid (XVII), which on heating in pyridine is decarboxylated to give the 5 (10)-dehydro derivative (XVIII).<sup>18</sup> Selective ketalization of (XVIII) at C-3 is accomplished by treatment with weak acid in methanol, yielding (XIX). Ethynylation at C-17 then gives the 3-dimethyl ketal of norethynodrel (XX). Weak acid cleavage of (XX) gives norethynodrel (VI), while more vigorous acid treatment gives norethindrone (VII). Conversion of (VII) to ethynodiol diacetate (IX) is accomplished as previously described.

FIG. 7: SYNTHESIS OF ETHYNODIOL DIACETATE

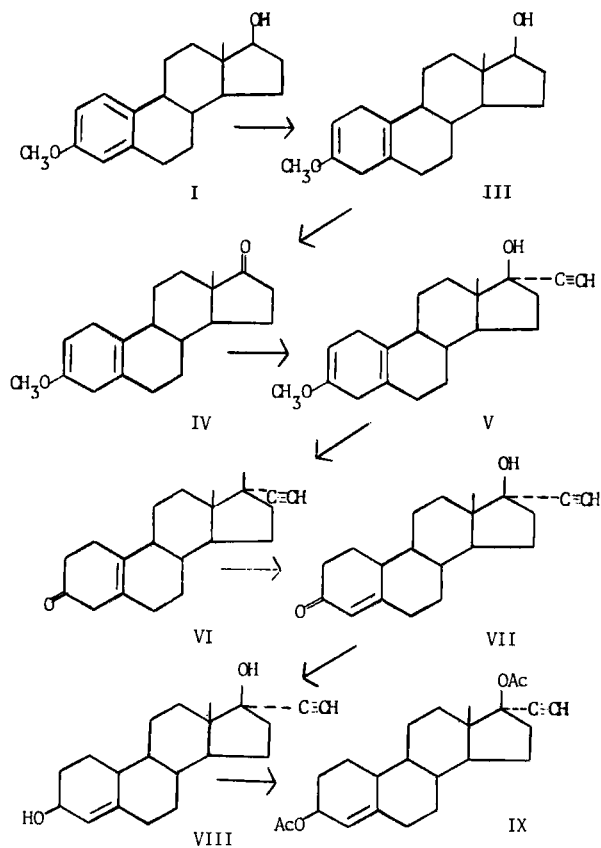


FIG. 8: SYNTHESIS OF ETHYNODIOL DIACETATE

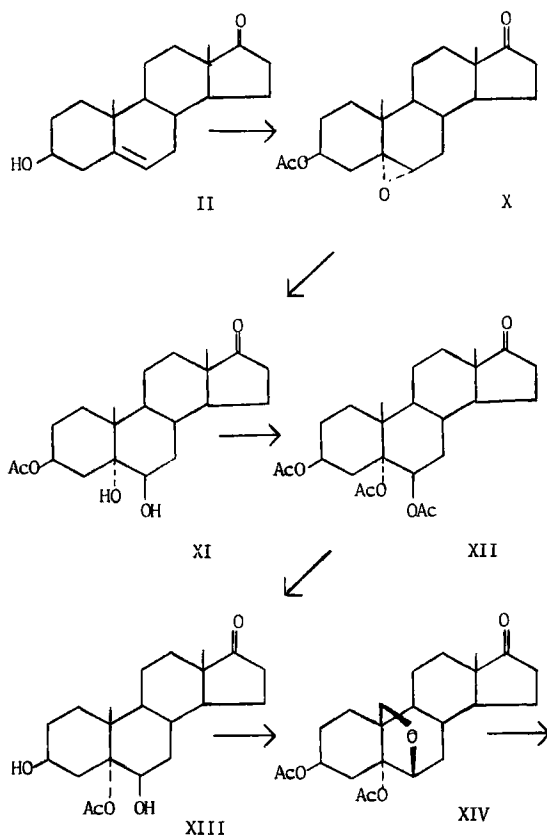
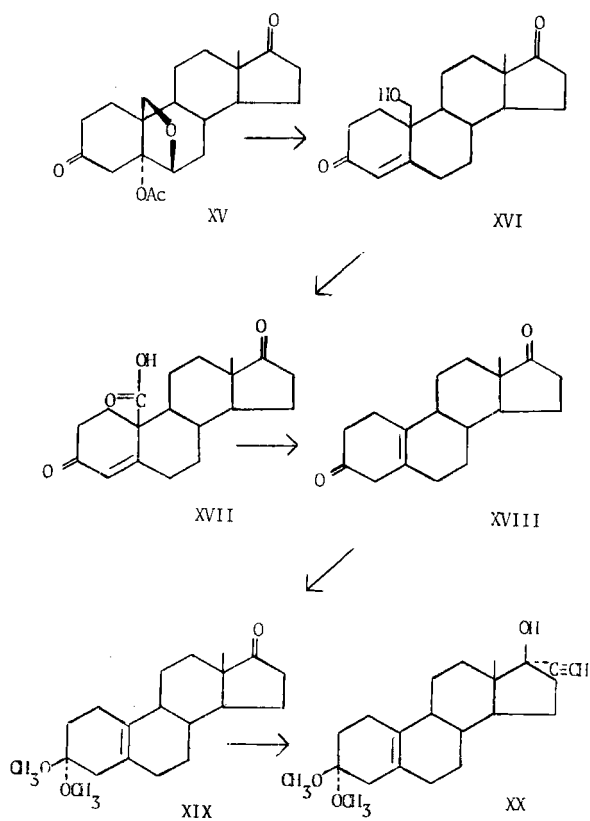


FIG. 8: (CONT.)



#### 4. Stability and Degradation

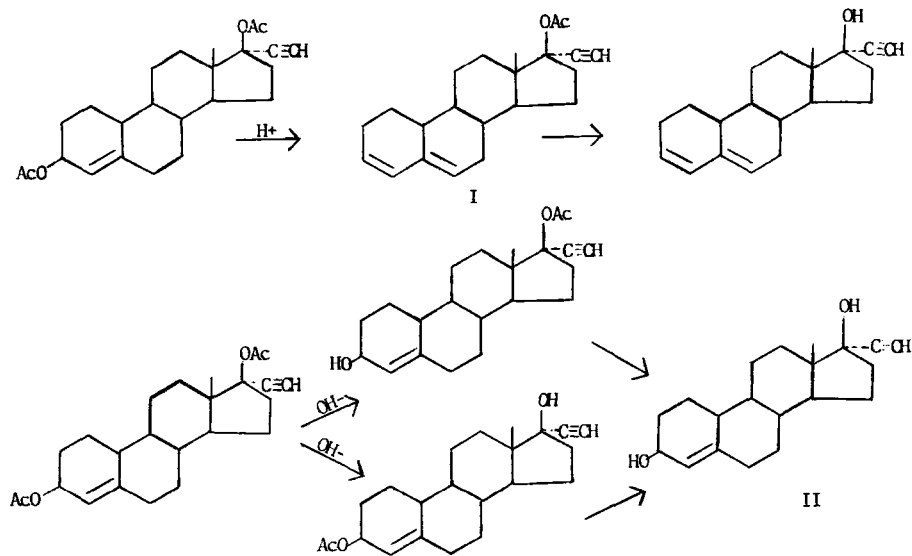
Ethynodiol diacetate appears to be very stable as a solid. The degradation of ethynodiol diacetate in both acidic and basic alcoholic solutions is shown in Figure 9. In the acidic alcohol solution, the primary degradation product was found to be the diene (I). In basic alcohol solution, the primary degradation product was found to be the diol (II).<sup>19</sup>

#### 5. Drug Metabolic Products and Pharmacokinetics

The major metabolites of ethynodiol diacetate in urine are shown in Figure 10. These metabolites were identified by Kishimoto, Kraychy, Ranney and Gantt.<sup>20</sup> The metabolism of ethynodiol diacetate by rat and human liver was reported by Freudenthal, Cook, Forth, Rosenfeld and Wall.<sup>21</sup> They found that the biotransformation reactions involved in the *in vitro* metabolism include deacetylation, saturation of ring A, aromatization of ring A, formation of 3-ketone and a  $\Delta^6$ -bond formation. A method of analysis of very low levels of the metabolite norethindrone has been developed by Freudenthal, Cook and Wall.<sup>22</sup> The principle of this method is to convert the cold norethindrone by enzyme reduction in the presence of NADPH-4<sup>3</sup>H to tritiated 17- $\alpha$ -ethynylestrane-3,17- $\beta$ -diol.

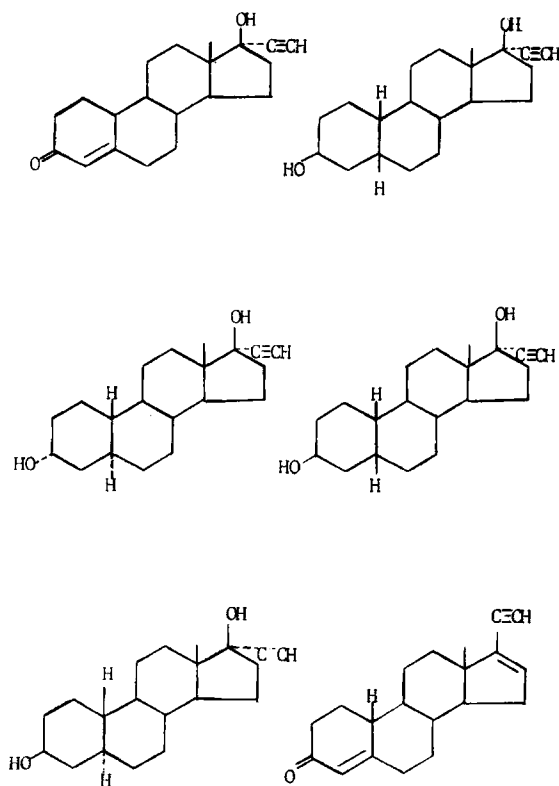
The pharmacokinetic profile of the total tritium label and metabolic composition in the plasma after an oral administration of ethynodiol diacetate-6,7-<sup>3</sup>H to a human subject was studied by Karim, Ranney, Cook and Bressler.<sup>23</sup> The absorption rate constant (k) of the total label was 0.79% <sup>3</sup>H per hour, the peak plasma level being attained after 3 hours. The elimination rate constant (K) of the total label was 0.0276% <sup>3</sup>H per hour (half-life 25 hours). The volume of distribution (V) was found to be 33L and the metabolic clearance rate (MCR) 21.9L per day. On chloroform extraction of the pooled plasma, 20% of the radioactivity was obtained as a free fraction which on TLC analysis gave two major spots tentatively identified as saturated dihydroxy metabolites and norethindrone. Eighty percent of the pooled plasma radioactivity was pres-

FIG. 9: DEGRADATION OF ETHYNODIOL DIACETATE IN ACIDIC & BASIC SOLUTION



# ETHYNODIOL DIACETATE

FIG. 10: MAJOR METABOLITES OF  
ETHYNODIOL DIACETATE IN URINE



ent as water-soluble conjugates which on acidic hydrolysis furnished two major aglycones having chromatographic mobilities similar to the two major spots. In a plasma sample taken one hour after administration of the labeled drug, 58% of the radioactivity was associated with the conjugated metabolites, 12.5% with the spot identified as saturated dihydroxy metabolites and 19.7% with the spot identified as norethindrone.

## 6. Methods of Analysis

### 6.1 Phase Solubility

Phase solubility analysis can be carried out by equilibrating the drug substance in hexane at 25°C. Figure 11 shows the phase solubility diagram of a reference standard run.<sup>24</sup>

### 6.2 Spectrophotometric Analysis

Ethynodiol diacetate does not have a useful spectrum for direct U.V. analysis. The solution of diene resulting from acid treatment has an absorbance maximum at about 236 nm. The USP XVIII assay is based on this reaction.<sup>6</sup>

### 6.3 Colorimetric Analysis

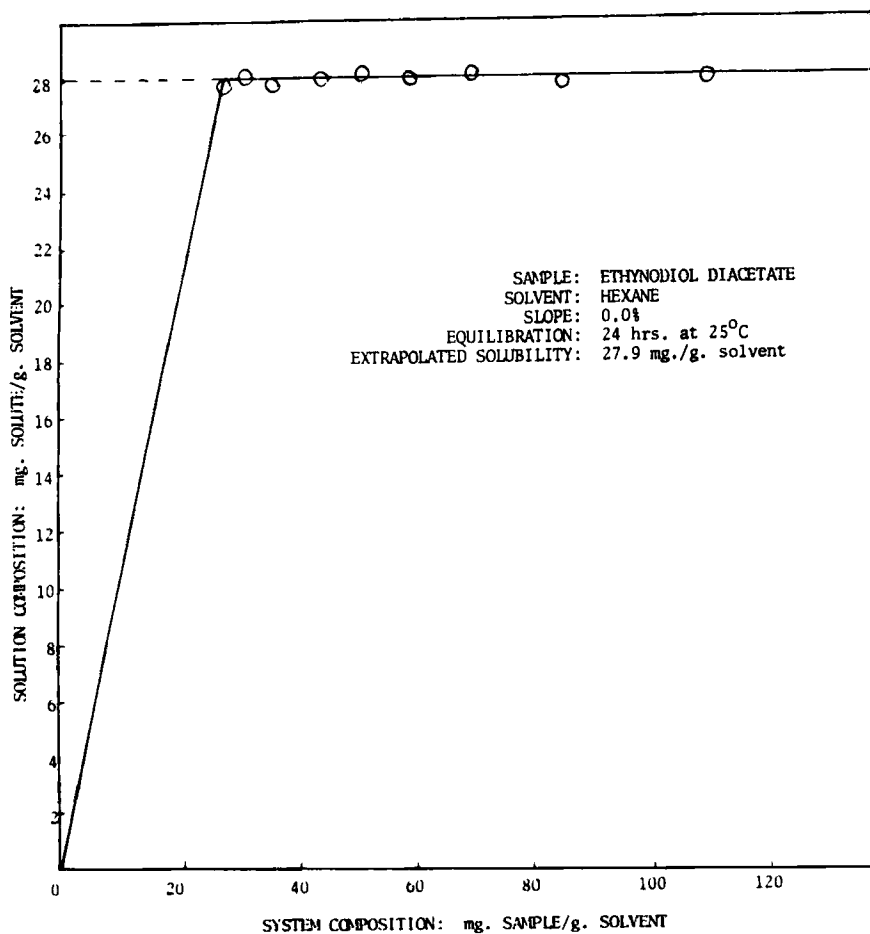
A variety of colorimetric methods have been developed to detect and to determine ethynodiol diacetate.

6.31 Reaction of ethynodiol diacetate with antimony trichloride in dry chloroform containing 1% acetic anhydride produces a violet color. The absorbance of the solution at 565 nm. is linear with ethynodiol concentration over a range of 5-60 mcg./5 ml. The method has been adapted for the analysis of ethynodiol dosage forms.<sup>25</sup> None of the other steroids commonly found in oral estrogen-progestin combination dosage forms interfere. A chloroform solution of antimony



ETHYNODIOL DIACETATE

FIG. 11:  
PHASE SOLUBILITY



trichloride has also been proposed as a spray reagent for ethynodiol diacetate in quantitative thin layer chromatography.<sup>26</sup>

- 6.32 Reaction of ethynodiol diacetate with 52% sulfuric acid for 5 minutes at room temperature yields a solution having an absorbance maximum at 484 nm.<sup>27</sup> This method may be used for quantitative determination of ethynodiol diacetate, provided that a preliminary separation from other steroids is made.
- 6.33 Reaction of ethynodiol diacetate with hydroxylamine hydrochloride and ferric chloride produces a deep red color which serves to distinguish the compound from steroids having no ester group. The color is not sufficiently stable for use in a quantitative determination.<sup>28</sup>

#### 6.4 Fluorometric Analysis

Ethynodiol diacetate can be quantitatively determined by fluorometry in 65% sulfuric acid solution, with an activation wavelength of about 458 nm, and measuring fluorescence at about 520 nm. Separation from other steroids is necessary due to their interference. The limit of sensitivity of the method is 4 mcg./100 ml.<sup>27</sup>

#### 6.5 Titrimetric Analysis

- 6.51 Ethynyl Titration - ethynodiol diacetate reacts stoichiometrically with silver nitrate in tetrahydrofuran. The nitric acid produced can be titrated with sodium hydroxide, either potentiometrically or using phenolphthalein as indicator.<sup>6</sup> One equivalent of the compound is titrated.
- 6.52 Ester Saponification - ethynodiol diacetate may be saponified with a known amount of standardized alcoholic potassium hydroxide.

## ETHYNODIOL DIACETATE

The excess base is then titrated with hydrochloric acid, either potentiometrically or using phenolphthalein as indicator.<sup>29</sup> One equivalent of the compound is saponified.

### 6.6 Chromatographic Analysis

6.61 Column Chromatography - the quantitative separation of ethynodiol diacetate and mestranol in dosage forms on Sephadex LH-20 has been reported.<sup>30</sup>

6.62 High Pressure Liquid Chromatography - ethynodiol diacetate can be separated from its possible degradation products and quantitatively determined by reverse-phase high pressure liquid chromatography, using a DuPont ODS column and methanol-water eluants.<sup>31</sup>

6.63 Thin Layer Chromatography - TLC systems and corresponding  $R_f$  values of ethynodiol diacetate are summarized in the following table:

#### Thin Layer Chromatography of Ethynodiol Diacetate

<u>Solvent System</u>	<u>Adsorbent</u>	<u>Detection</u>	<u><math>R_f</math></u>	<u>Reference</u>
cyclohexane: isopropanol (97:3)	SG	1, 2	0.40	32
benzene:methanol (95:5)	SG	1, 2	0.77	33
benzene:acetone (80:20)	SG	3, 4	0.68	33
chloroform: methanol (90:10)	SG	3, 4	0.76	33

methylene chlor- SG                      3, 4              0.84              33  
ide:methanol:water  
(150:9:0.5)

SG = Silica gel.

- Detection:
1. Spray with 50%  $H_2SO_4$ ,  
heat at 80°C for 10 minutes.
  2. Spray with phosphomolybdic  
acid.
  3. Spray with concentrated  
 $H_2SO_4$ ; heat at 100°C for  
30 minutes.
  4. Observe under short wave U.V.

#### 7. Acknowledgments

The authors wish to express their appreciation to Dr. N. W. Atwater, Dr. R. Bible, Dr. F. Colton, Mr. A. J. Damascus and Dr. J. Hribar for their help in preparing sections of the manuscript. The expert secretarial assistance of Miss Mia Mulder is also gratefully acknowledged, as is Mrs. Lorraine Wearley's aid in preparing the figures.

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**FLUDROCORTISONE ACETATE**

*Klaus Florey*



CONTENTS

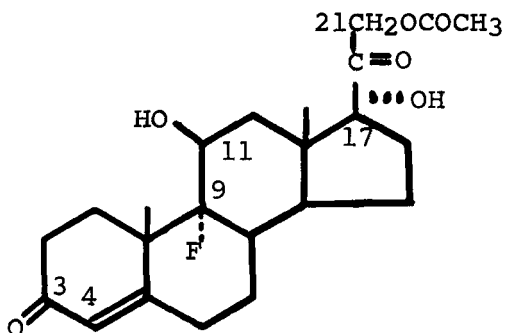
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## FLUDROCORTISONE ACETATE

### 1. Description

#### 1.1 Name, Formula, Molecular Weight

Fludrocortisone Acetate is 9 $\alpha$ -fluoro-11 $\beta$ , 17 $\alpha$ , 21-trihydroxy-4-pregnane-3, 20-dione, 21-acetate; also 9 $\alpha$ -fluorohydrocortisone acetate; 9 $\alpha$ -fluoro-17-hydroxycortisone 21-acetate; 9 $\alpha$ -fluorocortisol 21-acetate; fluodrocortisone 21-acetate; fluohydrisone, 21-acetate, fluohydrocortisone 21-acetate, SQ 9321.



C<sub>23</sub>H<sub>31</sub>FO<sub>6</sub>

M.W. 422.48

#### 1.2 Appearance, Color, Odor

Fludrocortisone Acetate is a white crystalline, odorless substance.

### 2. Physical Properties

#### 2.1 Infrared Spectra

Mesley<sup>1</sup> has reported four polymorphic forms and their infrared spectra.

Form A - as received from the British Pharmacopoeia.

Form B - evaporation of chloroform solution at room temperature followed by heating at 100° for 15 minutes.

Form C - (amorphous) - usually obtained by evaporation of chloroform or acetone solution

at room temperature.

Form D - may be obtained by spontaneous crystallization from form C.

These forms give the following characteristic absorption peaks ( $\text{cm}^{-1}$ )

Form A:

1412 1339 1274 1239 (sh) 1022 958 940 819 777 680

Form B:

1418 1339 1272 1226 1195 1020 958 945 868 782 678

Form C:

1418 (sh) 1267 1236 1198 1023 959 936 870 782 680

Form D:

1406 1344 1267 1232 1198 1020 955 942 869 - 678

The infrared frequencies of modifications and solvate described by Kuhnert-Brandstaetter and Gasser<sup>2</sup> (see also Section 2.9) are presented in Table 1.

Table 1  
9 $\alpha$ -Fluorohydrocortisone Acetate

Form	Frequencies ( $\text{cm}^{-1}$ )	
	OH	C=O and C=C
Modification I	3440 3350	1738, 1716, 1651, 1629(w)
II	3500(sh) 3460	1756, 1720, 1650, 1617
V	3510 3370 3300(sh)	1760, 1745, 1730, 1721 1650, 1611(w)
VI	3525 3500(w) 3350	1761, 1750, 1730, 1647
Methyl acetate solvate	3510 3320 3230(sh)	1761, 1748, 1738, 1722 1643
Ethyl acetate solvate	3515 3360	1759, 1738, 1725, 1652

# FLUDROCORTISONE ACETATE

Benzene solvate	3500	1761,1749,1736,1721, 1644
	3320	
Dimethylformamide solvate	3360	1740,1721,1660,1624
	3320(sh)	

The infrared spectrum of Squibb House Standard (batch #48004-001) in figure 1 represents modification I. (Form A)<sup>3</sup>

## 2.2 Nuclear Magnetic Resonance Spectrum

The NMR spectrum of fluodrocortisone acetate is presented in figure 2. The structural data presented in Table 2 agree with the assigned structure<sup>4</sup>. The two hydroxyl protons at C11 and C17 exchange with deuterium.

Table 2  
NMR Spectral Assignments of SQ 9321<sup>a</sup>

<u>Proton at</u>	<u>Chemical Shift, <math>\delta</math> (ppm)</u>
C4	5.64 s
C-11	4.10 b
C-18	0.77 s
C-19	1.49 s
C-21	4.78
C-21	5.06 ABq; J=17.0 Hz
C-21 Acetate	2.10
OH(Exchangeable)	5.00 b, 5.43 s

a = DMSO-d<sub>6</sub> s= singlet b= broad  
ABq = AB quartet

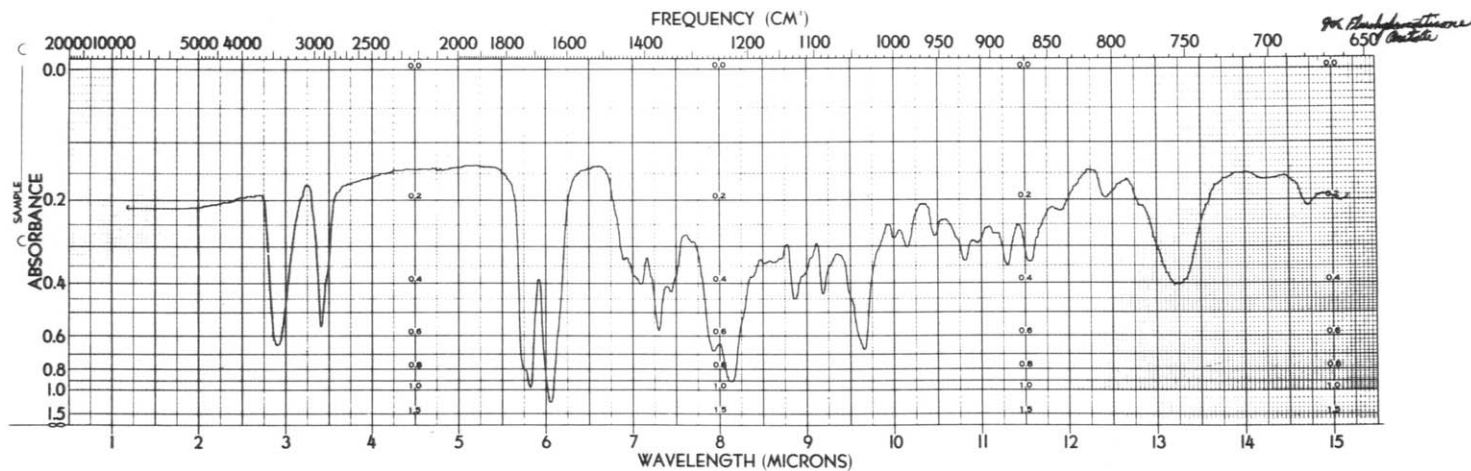


Figure 1. Infra Red Spectrum of Fludrocortisone Acetate (Squibb House Standard batch 48004-001) from KBr/Chloroform. Instrument:Perkin Elmer 21.

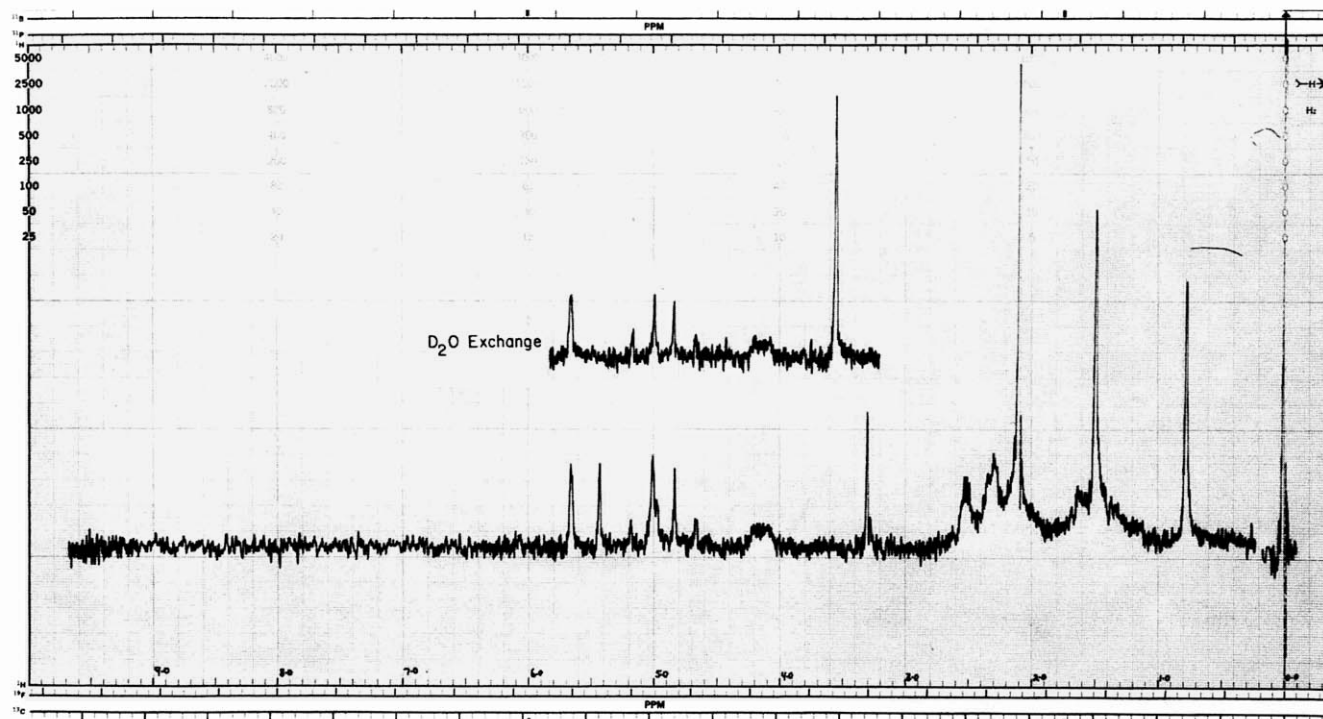


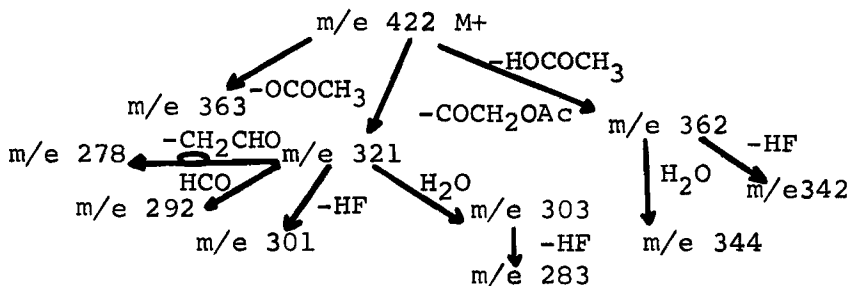
Figure 2. NMR Spectrum of Fludrocortisone acetate (batch 76682) in deuterated DMSO (Instrument:Varian XL-100).

### 2.3 Ultraviolet Spectrum

Fried and Sabo<sup>5</sup> reported  $\lambda$  max 238 nm;  
 $\epsilon = 16,800$  in ethanol.

### 2.4 Mass Spectrum

The low-resolution mass spectrum of SQ 9,321 (see figure 3) shows the expected  $M^+$  at  $m/e$  422. Corticosteroids generally show fragmentation patterns resulting from the loss of D-ring substituents (cf Analytical Profiles, Triamcinolone, Triamcinolone Acetonide, Triamcinolone 16, 17-diacetate). In addition, fluorinated steroids also have fragmentation pathways involving the loss of HF. Thus, the fragmentation pathways shown below depict the losses of these groups.



The base peak of  $m/e$  42 is from the acetyl portion of the 21-acetate. Although not as intense as those from A-ring dienones, the  $m/e$  121-123 ( $C_8H_9-11O$ ) and the  $m/e$  135-137 ( $C_8H_{11}-13O$ ) ions support the presence of the A-ring enone group. The mass spectrum is consistent with the proposed structure<sup>6</sup>.

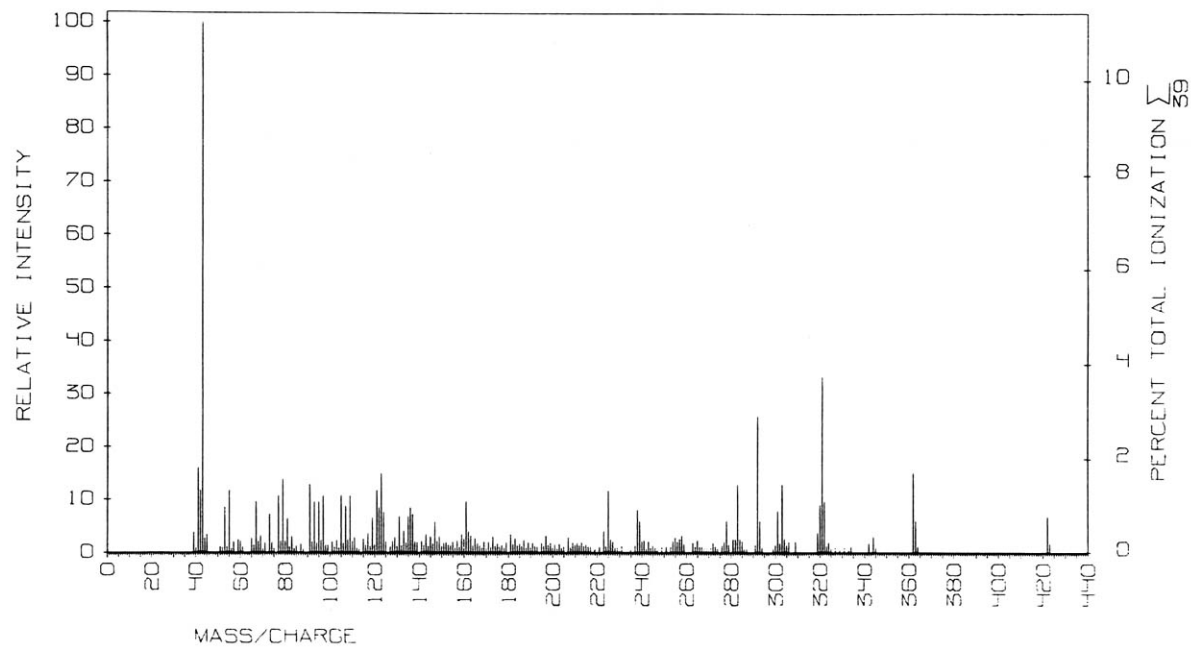


Figure 3. Low resolution mass spectrum of Fludorcortisone Acetate. (Squibb standard batch 48004-001) Instrument: AEI-MS-902.



2.5 Optical Rotation

$[\alpha]_D$	<u>Solvent</u>	<u>Ref</u>
+143°	Chloroform	5
+127°	Acetone	5
+149°	Dioxane	7
+145-150°	Dioxane	8

2.6 Melting Range

	<u>Ref</u>
233-234°	5
230° (decomp.)	7
220-233° (decomp.)	8

It was noted<sup>5</sup> that occasional samples started to melt at 205-208°, resolidified and eventually melted at 226-228 (See section 2.10). The melting behavior by the Kofler method has been described as follows<sup>9</sup>:

At 210° droplets start to form. The residual crystals grow to grains, squares, and hexagons that finally aggregate to a mosaic. Three or four different forms are produced at 160° in the glassy solidified melt. The bulk consists of long stalked spherulites of Form II that melt at 208-212° and leafy, partially fanlike radiate spherulites of Form III that melt at 205-208° and exhibit low-order interference colors. Form IV appears only rarely as fibrous-twisted spherulites. The melt becomes brown in color. The eutectic temperature with phenolphthalein is 202°. (For the melting behavior of polymorphic forms see also section 2.9.)

2.7 Differential Thermal Analysis

Squibb Standard (batch 48004-001) exhibits a sharp endotherm at 230°C<sup>10</sup>.

## 2.8 Solubility<sup>11</sup>

In water: 0.04 mg/ml; in acetone 56 mg/ml; in chloroform 20 mg/ml; in ether 4 mg/ml.

The dissolution behavior of crystalline fludrocortisone acetate and its pentanol and ethyl acetate solvates were studied by Shefter and Higuchi<sup>12</sup>. The initial dissolution rates of the solvate were significantly higher than the non-solvated form. Flynn determined the partition coefficient between ether and water as 45.7.<sup>13</sup>

## 2.9 Crystal Properties

The optical crystallographic properties of fludrocortisone acetate (probably modification A) and fludrocortisone itself have been presented as follows by Biles<sup>14</sup>.

	<u>System</u>	<u>Crystal Habit</u>	<u>Optic Sign</u>	<u>Axial Angle</u>
Fludrocortisone	Orthorhombic	Columnar	+	52°
Fludrocortisone acetate	Tetragonal	Columnar	-	0°
	<u>Optic Orientation</u>	<u>Refractive Indexes</u>		
		$\alpha$ ( $\omega$ )	$\beta$ ( $\xi$ )	$\gamma$
Fludrocortisone	XX    c YY    a ZZ    b	1.575	1.588	1.646
Fludrocortisone acetate	$\omega$    a $\xi$    c	1.604	1.538	--

Photomicrographs of the two crystals also were presented<sup>14</sup>.

The existence of several polymorphs has already been reported in section 2.1. As many as six may exist, according to Kuhnert-Brandstaetter and Gasser<sup>15</sup> but classification of the polymorphic conditions proved very difficult since except for modification I (m.p. 225-233°C), all other modifications show only very slight differences in their melting temperatures which are in the 205-215° range. Modification III and IV were not obtained in pure form. A further complication is the formation of solvates from a variety of solvents (see section 2.8). The powder X-ray diffraction pattern of fludrocortisone acetate (Form A) is presented in table 3<sup>16</sup>:

Table 3

$\frac{d}{\text{\AA}}$	Relative Intensity**	$d$	Relative Intensity
12.40 $\text{\AA}$	0.07	3.77	0.23
9.10	0.16	3.70	0.16
8.70	0.10	3.56	0.16
7.40	0.15	3.53	0.13
6.80	0.28	3.44	0.12
6.50	0.59	3.29	0.32
6.30	0.65	3.20	0.13
6.20	0.98	3.10	0.18
5.78	0.40	3.03	0.18
5.62	1.00	2.89	0.13
5.49	0.17	2.81	0.18
5.15	0.35	2.69	0.13
4.80	0.35	2.55	0.15
4.62	0.35	2.45	0.12
4.50	0.63	2.39	0.09
4.33	0.15	2.32	0.20
4.20	0.18	$*d = \frac{(\text{interplanar distance})n\lambda}{2 \sin \Theta}$	
4.12	0.29		
4.02	0.17		
3.94	0.12	**based on highest intensity of 1.00 Radiation: $K\alpha_1$ and $K\alpha_2$ Copper	
3.83	0.17		

Instrument: Phillips

### 3. Synthesis

Fludrocortisone Acetate (Fig. 4) was first synthesized by Fried and Sabo<sup>5</sup> by treatment of the epoxide III with hydrogen fluoride. Compound VII ( $\Delta^{8,14}$  hydrocortisone acetate) was found as a byproduct of the reaction<sup>5,7,42</sup>. Other approaches reported are introduction of the 4-double bond via bromination (IV and V), albeit in low yield<sup>17</sup>, and osmium tetroxide oxidation of the  $\Delta^{17(20)}$  precursor (VI)<sup>18</sup>. It can be purified from VII via the benzene adduct<sup>19</sup>. A method for the production of dense crystal has been patented<sup>20</sup>. It can be deacetylated to fluorohydrocortisone (II)<sup>5</sup>. It can serve as starting material for 9 $\alpha$ -fluoro-prednisolone (cf. ref. 21). For microbiological conversion to triamcinolone see section 5.2.

### 4. Stability-Degradation

Fludrocortisone acetate is very stable as a solid. In aqueous and alcoholic solutions the  $\alpha$ -ketol sidechain, as in all such corticosteroids, is prone to oxidative rearrangement and degradation at alkaline pH's.

It has been reported<sup>22</sup> that hydrocortisone and prednisolone, when exposed to ultraviolet light or ordinary fluorescent laboratory lighting in alcoholic solutions, undergo photolytic degradation of the A-ring. Since fludrocortisone acetate has the same A-ring as hydrocortisone it probably also is labile under these conditions.

### 5. Drug Metabolism

#### 5.1 Pharmacokinetics

The distribution in rat tissues and organs was studied with tritium labeled fludrocortisone<sup>23</sup>. The kinetics of metabolism were determined in man, dog, rat, monkey, and guinea

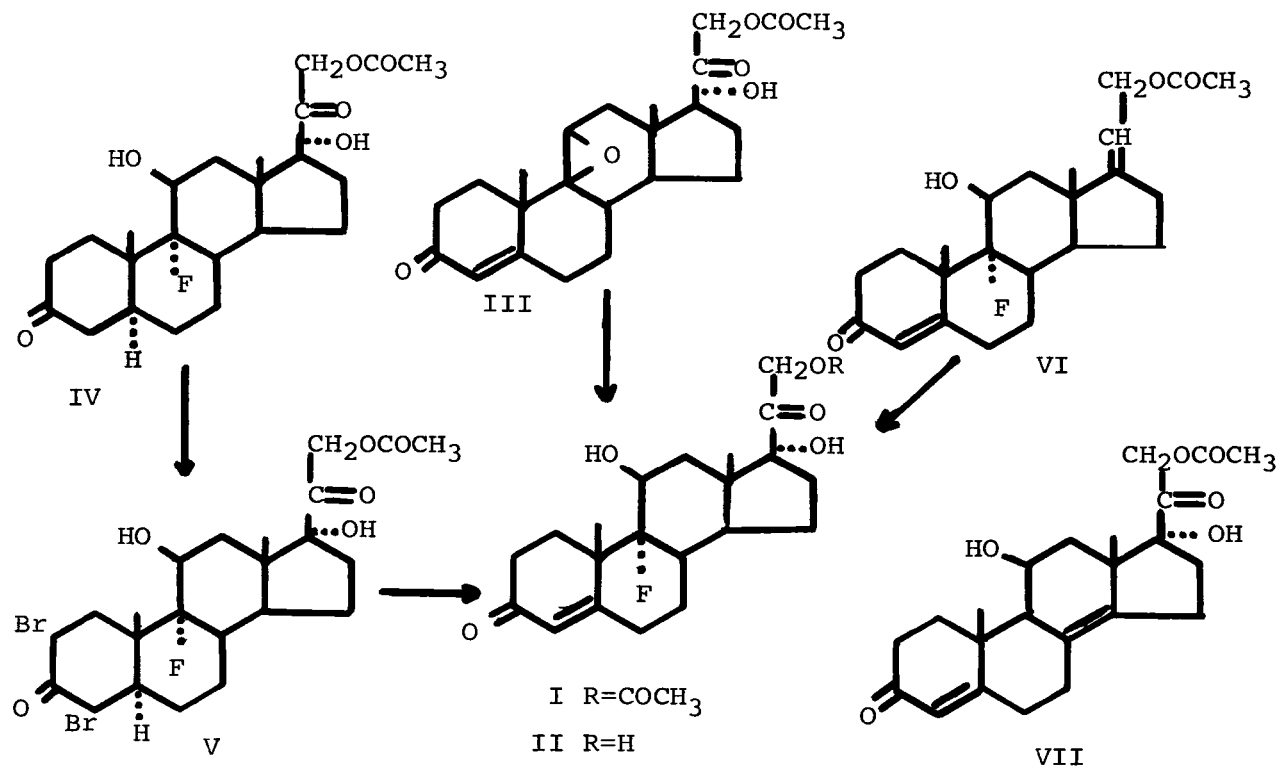


Figure 4

pig after I.V. and intraduodenal administration. Depending on species, 50% or more of the steroid remained unchanged 30 minutes after administration<sup>24</sup>. Fludrocortisone and its acetate had the same pharmacokinetic profile in dogs. The blood level reached a peak between 4 and 8 hours<sup>25</sup>. Silber<sup>26</sup> found that introduction of fluorine at position 9 prolonged the plasma half-life and depressed urinary excretion after oral and I.V. administration to dogs as compared to hydrocortisone. Disappearance of fludrocortisone acetate after incubation with rat liver slices<sup>27-31</sup> or perfusion of rat liver<sup>32</sup> was also studied.

## 5.2 Metabolic Products

After incubation of fludrocortisone with rat liver slices Schrievers<sup>33</sup> identified 9 $\alpha$ -fluoro-5 $\beta$ -pregnan-11 $\beta$ ,17 $\alpha$ , 21-trihydroxy-3,20-dione and 9 $\alpha$ -fluoro-5 $\beta$ -pregnan-3 $\beta$ , 11 $\beta$ ,17 $\alpha$ , 21-tetrahydroxy-20-one. There was no evidence for 5 $\alpha$ - or 20-hydroxy metabolites. Bush and Mahesh<sup>34</sup> identified the following metabolites in human urine:

9 $\alpha$ -Fluoro-3 $\alpha$ ,11 $\beta$ ,17 $\alpha$ ,20,21 pentahydroxy-5 $\beta$ -pregnane

9 $\alpha$ -Fluoro-3 $\alpha$ ,11 $\beta$ ,17 $\alpha$ ,20,21 pentahydroxy-5 $\beta$ -pregnane

9 $\alpha$ -Fluorotetrahydrocortisol

9 $\alpha$ -Fluoroallotetrahydrocortisol

9 $\alpha$ -Fluoro-20,20-dihydrocortisol

9 $\alpha$ -Fluorocortisol

9 $\alpha$ -Fluoro-11 $\beta$ -hydroxyetiocholanolone

9 $\alpha$ -Fluoro-11 $\beta$ -hydroxyandrostanone

Bush and Mahesh<sup>34</sup> noted the far greater proportion of 5 $\alpha$ -(H) steroids than found with the halogen-free parent steroid. The expected 11-ketone steroids were completely absent.

### 5.3 Microbiological Transformation

The following microbiological transformations of fludrocortisone and its acetate have been reported: 1-hydroxylation<sup>35</sup>, 1-dehydrogenation<sup>36,37</sup>, 6-hydroxylation<sup>38</sup>, 16-hydroxylation<sup>39</sup> (see also under Triamcinolone, Analytical Profiles of Drug Substances, Vol. 1), and 20-carbonyl reduction to 20-hydroxyl<sup>40</sup>. For transformation by mixed cultures see ref. 41.

## 6. Methods of Analysis

### 6.1 Elemental Analysis

<u>Element</u>	<u>% Theory</u>	<u>Reported</u> <sup>5</sup>
C	65.39	65.32
H	7.39	7.26
F	4.52	4.50

### 6.2 Direct Spectrophotometric Assay

The ultraviolet absorption band at 238 nm (see 2.3) is due to the  $\alpha,\beta$  unsaturated ketone of the A-ring. The absorbance is useful as a measure of purity from extraneous materials and has been so used<sup>8</sup>, albeit at 242 nm.

### 6.3 Colorimetric Methods

A number of colorimetric methods for identification, differentiation from other steroids and quantitation have been applied to fludrocortisone acetate. Based on reaction with the A-ring are the isoniazid<sup>43</sup> ( $\lambda$  max 382 nm in ethanol) and 2,4-dinitrophenylhydrazine<sup>30</sup> methods. Based on reduction of the dihydroxyacetone sidechain are the blue tetrazolium<sup>30</sup>, Porter-Silber<sup>30</sup>, and Nessler's reagent<sup>44</sup> methods. A blue chromogen ( $\lambda$  max 625 nm) is produced by reacting fludrocortisone acetate with 2,6-di-tert-butyl-p-cresol in alkaline solution<sup>45</sup>. Reactions with a phenol, hydroquinone, phosphoric-sulfuric acid mixture (amber color)<sup>46</sup>, p-nitro so-

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dimethylaniline ( $\lambda_{\text{max}}$  650 nm)<sup>47</sup> and a sulfuric acid, fructose, cysteine mixture ( $\lambda_{\text{max}}$  548 nm) have been described. The last reaction has also been used to determine residual fludrocortisone in fermentation broths.<sup>49</sup> Chromogens are also formed in concentrated sulfuric<sup>50</sup> and phosphoric acids.<sup>51</sup>

### 6.4 Polarographic Analysis

Cohen<sup>52</sup> subjected fludrocortisone to polarographic reduction in dimethylformamide and found two reducing waves:

	<u>Wave 1</u>	<u>Wave 2</u>
E 1/2 (Volts vs. mercury pool anode)	1.66	2.10
Id (Diffusion current constant)	1.4	1.8
n (Apparant number of electrons transferred)	0.03	0.69

### 6.5 Chromatographic Analysis

#### 6.51 Paper Chromatographic Analysis

For paper chromatographic systems, see Table 4.



Table 4

System #	Solvent System	Developing Time (hrs)	Rf Values	Ref.
1	Formamid/Chloroform	18	-	33
2	Methanol/Water/Benzene 1:1:2	4	-	33
3	Methanol/Water/Ethylacetate/ Benzene 25:25:2.5:47.5	4	-	33
4	Propylene glycol/Toluene	96	-	29
5	Benzene/Formamide	--	-	53
6	Toluene/Heptane, Methanol/Water 5:5:7:3	--	0.27	53
7	Benzene/Methanol/Water 2:1:1	--	0.9	53
8	Petroleum ether (b.p. 100-120°) Toluene/Methanol/Water 67:33:85:15	--	-	53
9	Benzene/Ethanol/Water 2:1:2	5	0.9	54
10	Toluene/Petr. ether (b.p. 30-60°), Methanol/Water 12:8:13:7	2-1/2	0.35	54
11	Benzene/Petr. ether (b.p. 90-100°), Methanol/Water 5:5:7:3	2-1/2	0.18	54
12	Methyl isobutyl ketone/Formamide 20:1	2-1/2	0.87	55

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The following detection systems have been reported:

<u>Detection Systems</u>	<u>Ref.</u>
U.V.	33
Tetrazolium	33,34
Phosphoric acid fluorescence	33
2,4 Dinitrophenylhydrazine	53
Tollen's Reagent	53
Isonicotinic acid hydrazide	55

System #12 can be used to separate fludro-cortisone acetate (Rf 0.87) from fludrocortisone (Rf 0.68) and 16 $\alpha$ -hydroxyfludrocortisone (Rf 0.30). It can be used for the quantitative determination of fludrocortisone acetate<sup>55</sup> by dissolving the ground tablets in dimethylformamide, spotting approx 100 mcg. on filter paper impregnated with formamide-methanol 20:80, developing with methyl isobutylketone-formamide 20:1, elution, reaction with isonicotinic acid hydrazide and determination of the absorbance at 415 nm against a standard, using the general procedure of Roberts and Florey<sup>56</sup>.

### 6.52 Thin Layer Chromatographic Analysis

Experience with the thin layer chromatography of fludrocortisone acetate is summarized in Table 5.

Table 5  
Rf or "running distance" values  
(for explanation of individual values, see below)

<u>System</u>	<u>1</u>	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>E</u>	<u>a</u>	<u>b</u>	<u>c</u>
Fludrocortisone Acetate	0.87	1.03	1.14	1.16	2.6	0.62	0.48	0.42	0.31
Fludrocortisone	-	0.24	0.33	0.63	0.70	0.01	--	--	--
<u>System</u>	<u>I</u>	<u>II</u>	<u>III</u>	<u>IV</u>	<u>V</u>	<u>VI</u>			
Fludrocortisone Acetate	--	--	--	--	0.55	0.90			
Fludrocortisone	0.49	0.46	0.29	0.71	--	0.76			

System 1<sup>57</sup>: Kieselguhr G plate; Dichloroethane/methylacetate/water 2:1:1;  
Spray reagent: Alkaline 2,5-diphenyl-3(4-styrylphenyl)tetrazolium solution;  
"Running distances" values related to cortisone acetate = 1.00;  
Systems A-E<sup>58</sup>: Kieselguhr GF 254 plates; Spray reagent: Tetrazolium blue;  
"Running distance" values: A, B, C, E related to hydrocortisone acetate = 1.00  
D related to hydrocortisone = 1.00

Solvent systems: A- 1,2-Dichloroethane:methanol:water 95:5:0.2  
B- 1,2-Dichloroethane:2-methoxyethyl acetate:water 80:20:1  
C- Cyclohexane:ethylacetate:water 25:75:1  
D- Stationary phase: 20% v/v formamide in acetone  
Mobile phase: Chloroform:ether:water 80:20:0.5  
E- Stationary phase: 25% v/v formamide in acetone  
Mobile phase: Cyclohexane:tetrachloroethane:water  
50:50:0.1

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Systems a-c<sup>59</sup>: Kieselguhr G plates; Spray reagent: Tetrazolium blue. Values given are R<sub>f</sub> values.

Solvent systems: a - methylene chloride:toluene  
60:40  
b - methylene chloride:toluene  
50:50  
c - chloroform:toluene 25:75

Systems I - v<sup>60</sup>:

Silica gel plates, Spray reagent: Vanillin-perchloric acid sprayed over tetrazolium reagent. Values given are R<sub>f</sub> values.

Solvent system I - Ethylacetate  
II - Methylene chloride:dioxan:  
water  
III - Chloroform-ether-water  
(80:20:0.5) on formamide  
plate  
IV - Amylacetate-acetone 1:1  
V - Ether

System VI<sup>55</sup>:

Silica gel GF Plate, U.V. detection or elution and reaction with Nydrazid.

Solvent: Ether-dimethylformamide, acetone, methanol 88:8:2:2. In this system  $\Delta^{8,14}$ -hydrocortisone acetate has an R value of 0.83 in relation to fludrocortisone acetate.

### 6.6 Bioassay

A sensitive bioassay is based on the urinary Na<sup>+</sup>/K<sup>+</sup> ratio, expressed as percent of the control value after injection of fludrocortisone acetate into adrenalectomized rats<sup>61</sup>.

### 6.7 Other

Bismuth oxidation to the corresponding 17-ketosteroid has also been used as the basis for an analytical method<sup>62</sup>.

### 7. Determination in Body Fluids and Tissues

References mentioned earlier, can be summarized as follows:

	References :
Thin Layer Chromatography	2
Paper Chromatography	29, 33, 34
Colorimetric	25, 30, 31, 49
Bioassay	61

### 8. Determination in Pharmaceutical Preparations

The following references specifically mention analysis in pharmaceuticals.

	References :
Paper Chromatography	55
Colorimetric	8, 45, 47

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Literature surveyed through July 1972.

## **FLURAZEPAM HYDROCHLORIDE**

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Chemistry reviewed by R. I. Fryer.

INDEX

Analytical Profile - Flurazepam Hydrochloride

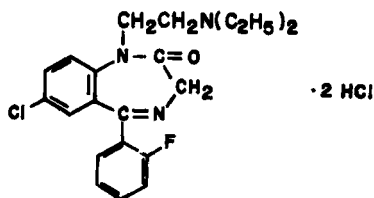
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# FLURAZEPAM HYDROCHLORIDE

## 1. Description

### 1.1 Name, Formula, Molecular Weight

Flurazepam hydrochloride is 7-chloro-1-(2-[diethylamino]ethyl)-5-(o-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one dihydrochloride.



$C_{21}H_{23}ClFN_3O \cdot 2HCl$

Molecular Weight: 460.83

### 1.2 Appearance, Color, Odor

Flurazepam hydrochloride occurs as an off-white to yellow, nearly odorless, crystalline powder.

## 2. Physical Properties

### 2.1 Infrared Spectrum

The infrared spectrum of flurazepam hydrochloride is presented in Figure 1 (1). The spectrum was measured with a Perkin-Elmer 621 Spectrophotometer in a KBr pellet containing 1.0 mg of flurazepam hydrochloride/300 mg of KBr. The assignments for the characteristic bands in the infrared spectrum are listed in Table I (1).

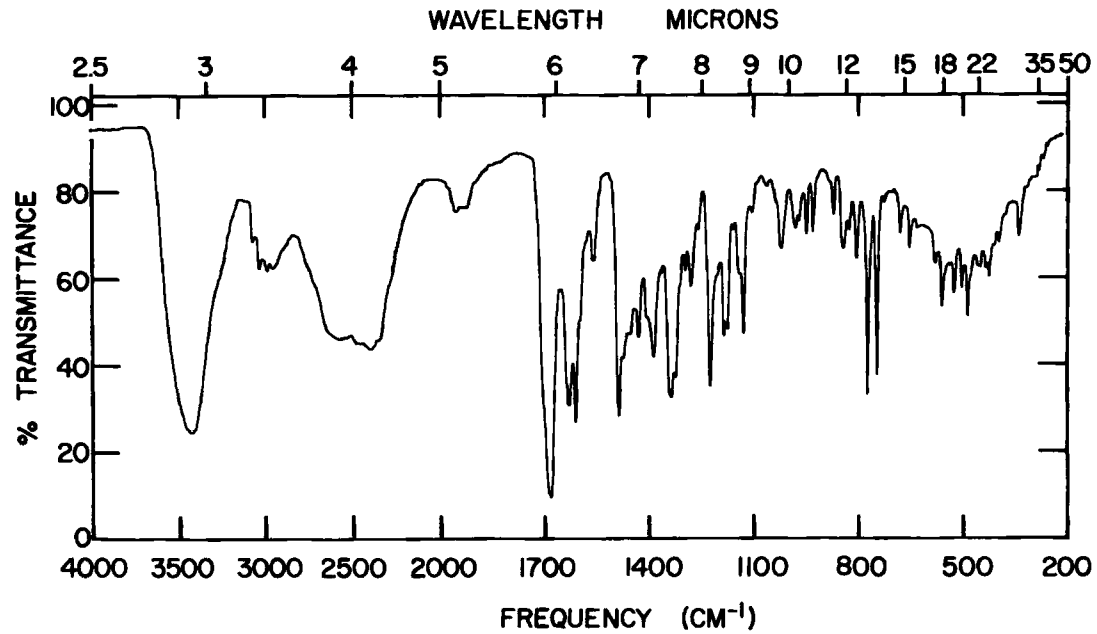
Table I

#### Infrared Assignments for Flurazepam Hydrochloride

<u>Frequency (cm<sup>-1</sup>)</u>	<u>Characteristic of</u>
3066	aromatic CH stretching vibrations
2500	hydrochloride of tertiary amine
1683	C=O stretching vibrations
1560 and 1483	aromatic ring
748	4 adjacent H's on phenyl ring

Figure 1

Infrared Spectrum of Flurazepam Hydrochloride



## 2.2 Nuclear Magnetic Resonance Spectrum (NMR)

### 2.21 Proton Spectrum

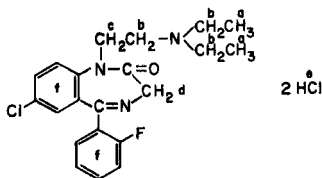
The proton NMR spectra shown in Figure 2 were run on a Jeolco 60 MHz NMR using tetramethylsilane as an internal reference (2). The flurazepam hydrochloride spectrum, Figure 2A, was obtained by dissolving 59.0 mg of sample in 0.5 ml of methanol- $d_4$ . The spectral assignments are listed in Table II (2). The solvent peak for methanol- $d_4$  occurs about 3.27 ppm and interferes with the assignments in that region. Therefore, the spectrum of flurazepam base (54.2 mg/0.5 ml  $CDCl_3$ ), shown in Figure 2B, was determined and the spectral assignments presented in Table II (2).

### 2.22 <sup>19</sup>F Spectrum

The <sup>19</sup>F spectrum shown in Figure 2C was obtained with a Jeolco C-60 HL instrument with a <sup>19</sup>F module crystal modified to a frequency of 56.446 MHz. Two hundred mg of flurazepam hydrochloride were dissolved in 0.5 ml of methanol containing  $CCl_3F$  as the internal reference (2). The spectrum consists of a quintet at -108 ppm. The choice of  $CCl_3F$  as the internal reference along with the assignment of -108 ppm is in accordance with Bovey (3).

Table II

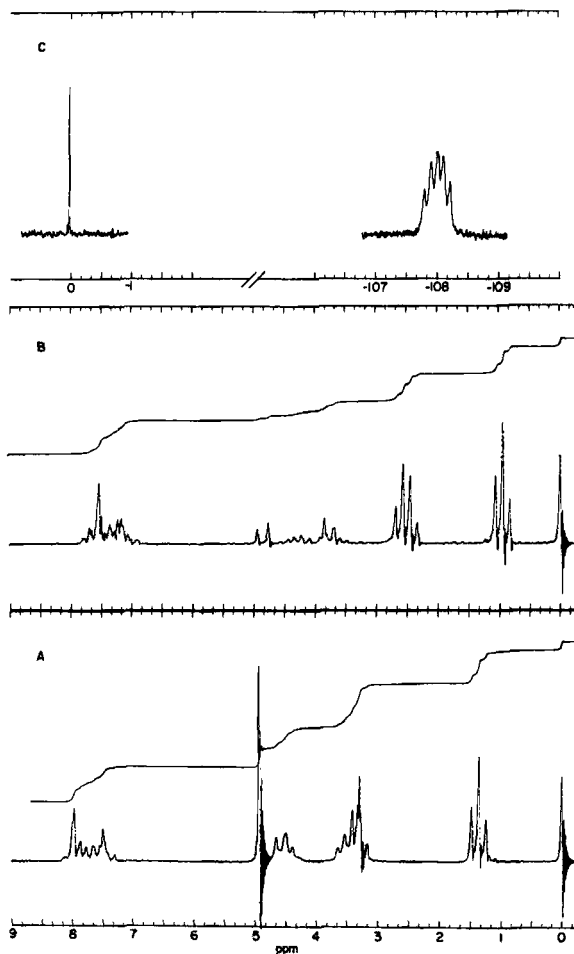
NMR Assignments for Flurazepam and Flurazepam Hydrochloride



<u>Proton</u>	<u>No. of Protons</u>	<u>Chemical Shift (ppm)</u>	<u>Multiplicity</u>
<u>Flurazepam Hydrochloride</u>			
a	6	1.40	Triplet ( $J_{H_a-H_b} = 7\text{Hz}$ )
b	6	~3.42	Multiplet
c	2	~4.50	Triplet

Figure 2

- A. NMR Spectrum of Flurazepam Hydrochloride
- B. NMR Spectrum of Flurazepam Base
- C.  $^{19}\text{F}$  NMR Spectrum of Flurazepam Hydrochloride



# FLURAZEPAM HYDROCHLORIDE

<u>Proton</u>	<u>No. of Protons</u>	<u>Chemical Shift (ppm)</u>	<u>Multiplicity</u>
d	2	~4.50	
e	2*	4.92	Singlet
f	7	7.30-8.15	Multiplet
<u>Flurazepam Base</u>			
a	6	0.95	Triplet ( $J_{H_a-H_b} = 7.5\text{Hz}$ )
b	6	2.50	Quartet ( $J_{H_b-H_a} = 7.5\text{Hz}$ )
c	2	3.47-4.55	Multiplet
d	2	3.76-4.85	Two sets of doublets ( $J = 11\text{ Hz}$ )
f		6.95-7.80	Multiplet

$^{19}\text{F}$  Spectrum of Flurazepam Hydrochloride -108 Quintet

\* Also any  $\text{H}_2\text{O}$  present in methanol- $\text{d}_4$

## 2.3 Ultraviolet Spectrum (UV)

When the UV spectrum of flurazepam hydrochloride was scanned from 450 to 210 nm, three maxima and three minima were observed. The maxima are located at 362 nm ( $\epsilon = 3.7 \times 10^3$ ), 284 nm ( $\epsilon = 1.2 \times 10^4$ ), and 239 nm ( $\epsilon = 2.8 \times 10^4$ ). The minima occur at 333 nm, 263 nm, and 219 nm. The spectrum shown in Figure 3 was obtained from a solution of 1.006 mg of flurazepam hydrochloride/100 ml of acidified methanol (2.8 ml of concentrated  $\text{H}_2\text{SO}_4$  diluted to 100 ml with anhydrous methanol)(4).

## 2.4 Fluorescence Spectrum

The excitation and emission spectra for flurazepam hydrochloride (1 mg/ml of methanol) are shown in Figure 4 (5). One maximum appears in the excitation spectrum at 378 nm and one maximum in the emission spectrum at 492 nm.

## 2.5 Mass Spectrum

The mass spectrum of flurazepam hydrochloride was obtained using a CEC 21-110 mass spectrometer with an ionizing energy of 70 ev. The output from the mass spectrometer was analyzed and presented in the form of a bar



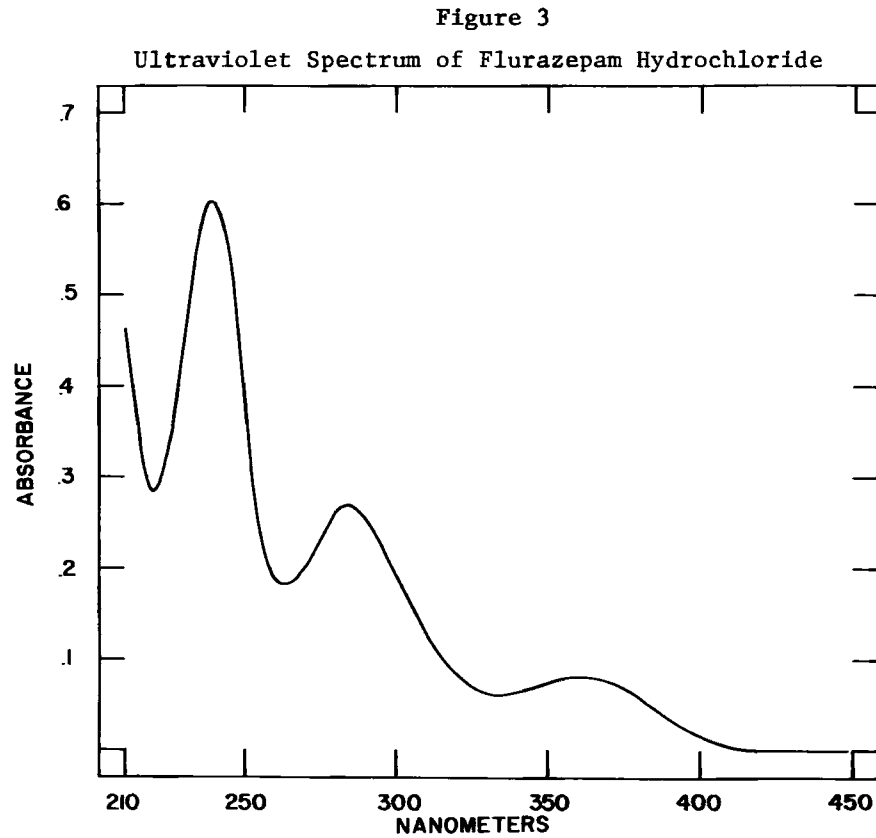


Figure 4

Fluorescence Spectra of Flurazepam Hydrochloride

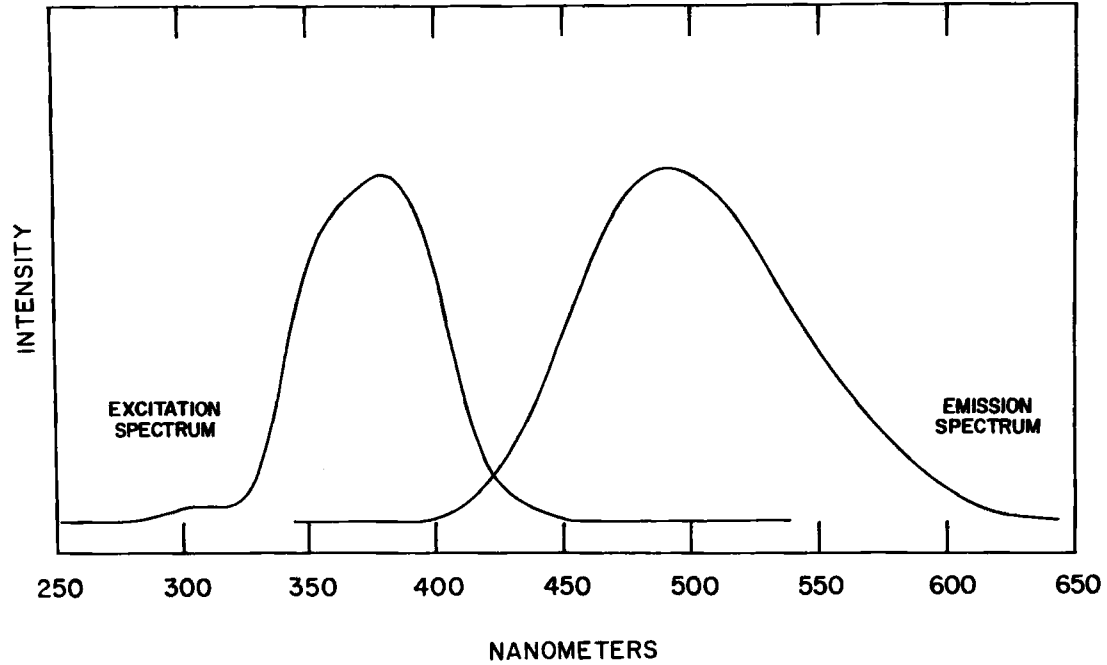
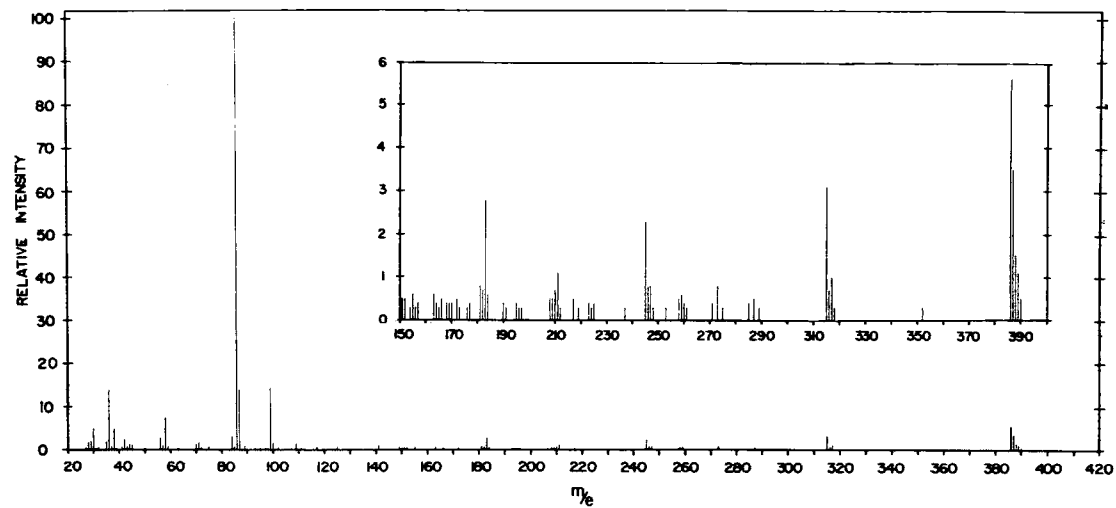


Figure 5  
Mass Spectrum of Flurazepam Hydrochloride



graph, shown in Figure 5, by a Varian 100 MS dedicated computer system. Due to the extreme intensity of the base peak at  $m/e$  86, the relative intensity of the peaks at the higher mass units are very weak. Therefore, the peaks from  $m/e$  150 up were subjected to a ten fold scale expansion (Figure 5 - insert)(6). The parent peak ( $M^+$ ) at  $m/e$  387 is due to the free flurazepam base. The base peak at  $m/e$  86 is due to the  $(C_2H_5)_2NCH_2$  fragment. The other major peaks can be attributed to stepwise fragmentation of the parent ion; i.e.,  $M^+ - (C_2H_5)_2N = 315$ ,  $315 - CH_2N = 287$  (6).

#### 2.6 Optical Rotation

Flurazepam hydrochloride exhibits no optical activity.

#### 2.7 Melting Range

Flurazepam hydrochloride melts with decomposition within a  $5^\circ$  range between  $208^\circ$  and  $218^\circ C$  when the USP XVIII Class Ia procedure is used (7).

#### 2.8 Differential Scanning Calorimetry (DSC)

The thermal properties of flurazepam hydrochloride in the melting region are very dependent on previous thermal history. Using a temperature program of  $20^\circ C/min.$ , the extrapolated onset of an endothermic transition occurred at  $215.8^\circ C$  followed immediately by the exothermic transition due to decomposition at  $229.5^\circ C$  (Figure 6). At a scan rate of  $10^\circ C/min.$ , a small endothermic transition occurs at  $203.3^\circ C$  followed by sample decomposition at  $217.5^\circ C$ . Due to the sample instability in the region of the melt, the  $\Delta H_f$  was not determined (8).

#### 2.9 Thermal Gravimetric Analysis (TGA)

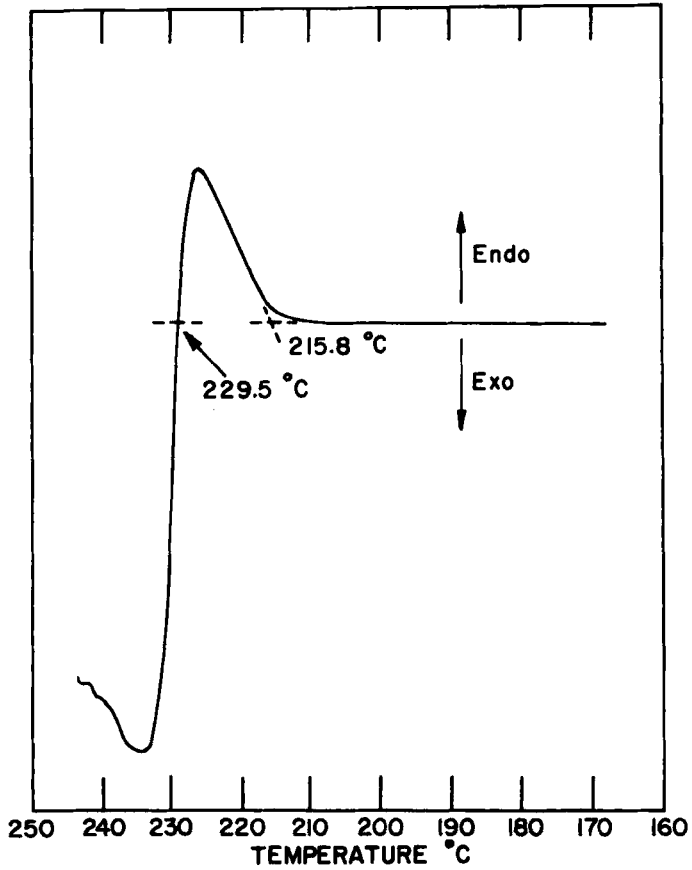
A TGA performed at a scan rate of  $10^\circ C/minute$  showed little weight loss for flurazepam hydrochloride from ambient to  $190^\circ C$ . A weight loss amounting to about 70% of the sample occurred between 190 and  $345^\circ C$  (8).

#### 2.10 Solubility

The solubility data for flurazepam hydrochloride obtained by equilibration for 20 hours at  $25^\circ C$  are given in Table III (9).

Figure 6

DSC Scan for Flurazepam Hydrochloride



# FLURAZEPAM HYDROCHLORIDE

Table III

## Solubility Profile for Flurazepam Hydrochloride

<u>Solvent</u>	<u>Solubility (mg/ml)</u>
3A alcohol	28.3
benzene	0.4
chloroform	11.1
95% ethanol	260
diethyl ether	0.2
methanol	340
petroleum ether (30°-60°)	0.2
2-propanol	14.6
water	>500

### 2.11 X-ray Crystal Properties

The x-ray powder diffraction pattern of flurazepam hydrochloride is presented in Table IV (10). The instrumental conditions are given below.

#### Instrumental Conditions

##### General Electric Model XRD-6 Spectrogoniometer

Generator:	50KV-12-1/2 MA
Tube target:	Copper
Radiation:	Cu $K_{\alpha}$ = 1.542 Å
Optics:	0.1° Detector slit
	M.R. Soller slit
	3° Beam slit
	0.0007 inch Ni filter
	4° take off angle
Goniometer:	Scan at 0.2° 2θ per minute
Detector:	Amplifier gain - 16 course,
	8.7 fine
	Sealed proportional counter
	tube and DC voltage at
	plateau
	Pulse height selection $E_L$ -
	5 volts; Eu - out
	Rate meter T.C. 4
	2000 C/S full scale
Recorder:	Chart Speed - 1 inch per 5
	minutes

Samples prepared by grinding at room temperature.

Table IV  
X-ray Powder Diffraction Pattern of  
Flurazepam Hydrochloride

$2\theta$	$d(\text{\AA})^*$	$I/I_0^{**}$	$2\theta$	$d(\text{\AA})^*$	$I/I_0^{**}$
8.32	10.6	3	31.40	2.85	1
9.04	9.78	20	32.36	2.77	38
11.62	7.62	37	32.92	2.72	7
12.49	7.09	15	33.30	2.69	8
12.86	6.88	11	33.88	2.65	6
15.50	5.72	62	34.24	2.62	7
16.40	5.40	11	34.87	2.57	11
16.78	5.28	9	35.20	2.55	14
17.38	2.10	7	35.77	2.51	5
17.94	4.94	5	36.18	2.48	10
18.65	4.76	50	37.28	2.41	5
19.16	4.63	100	37.72	2.38	11
20.05	4.43	26	38.17	2.36	11
20.38	4.36	20	38.78	2.32	19
20.75	4.28	19	39.47	2.28	3
21.66	4.10	12	40.18	2.24	3
23.14	3.84	82	40.86	2.21	5
23.61	3.77	39	41.28	2.19	2
24.32	3.66	40	41.86	2.16	5
24.50	3.63	36	42.20	2.14	8
25.18	3.54	98	42.94	2.11	9
25.96	3.43	12	43.58	2.08	9
26.18	3.40	33	44.24	2.05	4
26.40	3.38	24	44.70	2.03	5
26.96	3.31	12	46.06	1.97	7
27.44	3.25	15	47.00	1.93	4
27.94	3.19	18	47.52	1.91	2
28.46	3.14	5	48.32	1.88	5
28.78	3.10	5	49.08	1.86	3
29.96	2.98	19	50.00	1.82	3
30.54	2.93	4	50.26	1.82	3
31.00	2.88	15	50.84	1.80	4

\* $d$  - (interplanar distance)  $\frac{n\lambda}{2 \sin \theta}$

\*\* $I/I_0$  = relative intensity (based on highest intensity of 1.00)

### 2.12 Dissociation Constant

The pKa's for flurazepam were determined spectrophotometrically and found to be  $1.90 \pm 0.05$  and  $8.16 \pm 0.05$  (11). The apparent pKa<sub>2</sub> has also been determined from the titration curve in a 2-propanol:water (1:1) mixture and found to be  $7.0 \pm 0.1$  (11). In water, the trialkylamino type compounds are stronger bases, on the average, by 0.9 pK units (11,12). Therefore, the estimated pKa<sub>2</sub> in water would be 7.9 which is in good agreement with that found spectrophotometrically.

### 3. Synthesis

Flurazepam hydrochloride may be prepared by the reaction scheme shown in Figure 7. 7-Chloro-5-(o-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one is reacted with diethylaminoethyl chloride in the presence of sodium methoxide to yield flurazepam which is then converted to flurazepam hydrochloride by the addition of hydrochloric acid (13). A complete review of the chemistry of benzodiazepines by Archer and Sternbach presents several pathways to arrive at the basic benzodiazepine (14).

### 4. Stability Degradation

When sealed amber ampuls with dilute solutions of flurazepam hydrochloride in 0.1N HCl, water, and 0.1N NaOH:3A alcohol (1:1) were heated in a boiling water bath for one hour, the degradation products shown in Figure 8 were observed by thin layer chromatography (15). In 0.1N HCl solution the main hydrolysis product was 5-chloro-2-(2-diethylaminoethylamino)-2'-fluorobenzophenone hydrochloride. In aqueous solution the main degradation product was 7-chloro-5-(o-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one. Finally, in the 0.1N NaOH:3A alcohol (1:1) solution, the main degradation products were 5-chloro-2-(2-diethylaminoethylamino)-2'-fluorobenzophenone and 2-chloro-10-(2-diethylaminoethyl)-9-acridone. When a solution of flurazepam hydrochloride in water is irradiated with light from a high pressure U.V. lamp for 3 hours, some hydrolysis to 5-chloro-2-(2-diethylaminoethylamino)-2'-fluorobenzophenone occurs (16). Flurazepam hydrochloride, when stored in well closed, light resistant containers, is quite stable.



Figure 7

## Synthesis for Flurazepam Hydrochloride

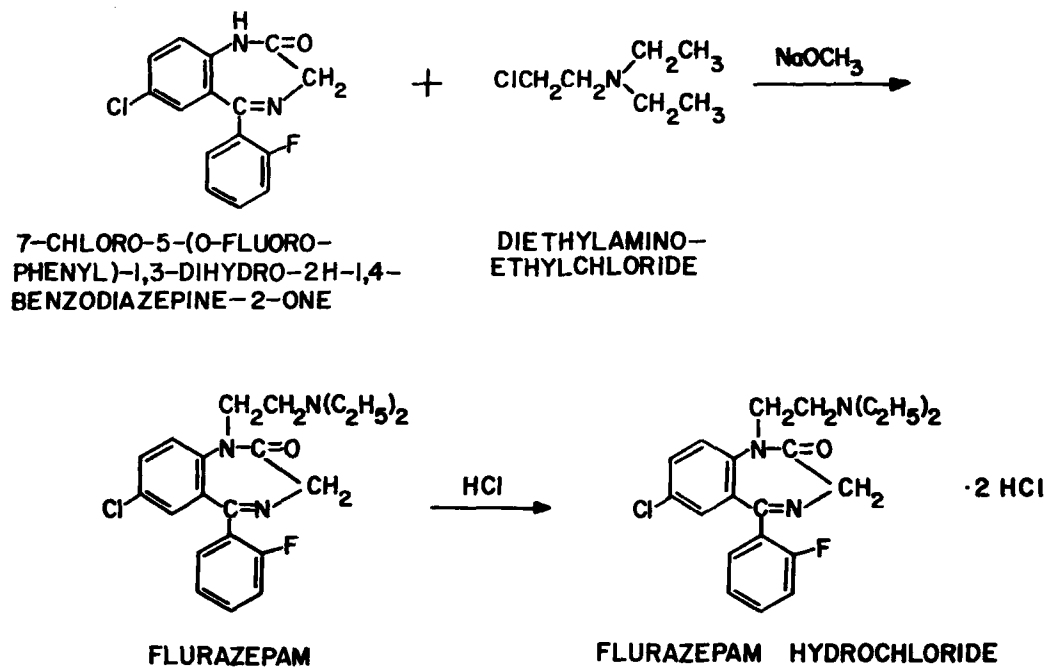
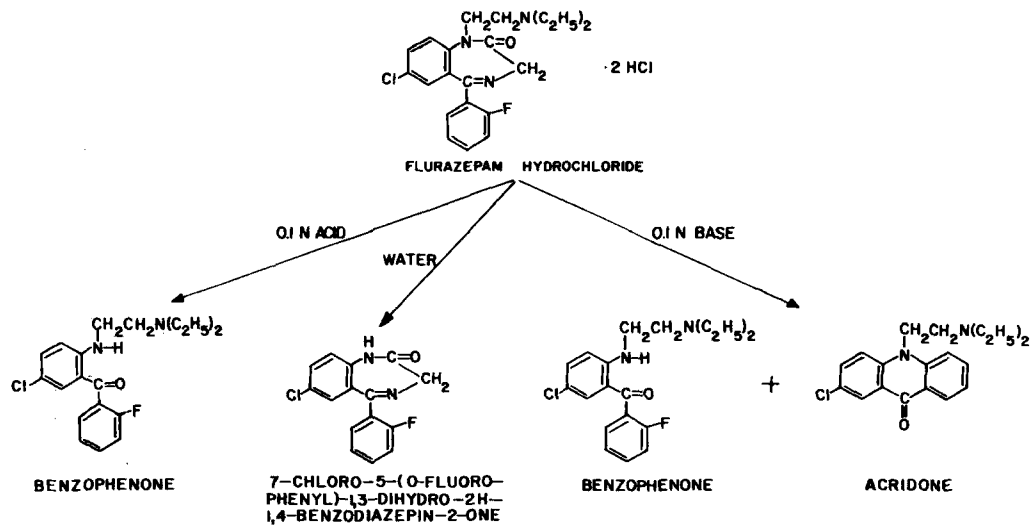


Figure 8

Major Degradation Products for Flurazepam Hydrochloride  
in Acid, Basic, and Aqueous Solutions



## 5. Drug Metabolic Products

The metabolic pathways of flurazepam in dog and man are shown in Figure 9 (17-22). Initially, five metabolites plus the intact drug were found in the urine of dogs. Four of these metabolites were identified as monodesethyl-flurazepam, didesethylflurazepam, flurazepam-N<sub>1</sub>-ethanol, and N<sub>1</sub>-desalkyl-3-hydroxyflurazepam (17,20). The fifth metabolite was tentatively identified as a phenolic derivative of N<sub>1</sub>-desalkyl-flurazepam by mass spectrometry (17). The major metabolite found in the dog urine is flurazepam-N<sub>1</sub>-acetic acid (17,19). In human urine, the flurazepam-N<sub>1</sub>-ethanol was the major metabolite along with smaller quantities of the mono- and didesethyl-flurazepam and N<sub>1</sub>-desalkyl-3-hydroxyflurazepam (17-22).

## 6. Methods of Analysis

### 6.1 Elemental Analysis

The results from the elemental analysis are listed in Table V (23).

Table V

#### Elemental Analysis of Flurazepam Hydrochloride

<u>Element</u>	<u>% Theory</u>	<u>% Found</u>
C	54.74	54.73
H	5.47	5.46
N	9.12	9.11
F	4.12	4.22
Cl	7.69	7.86
Cl <sup>-</sup> (ionic)	15.38	15.42

### 6.2 Fluorine Analysis

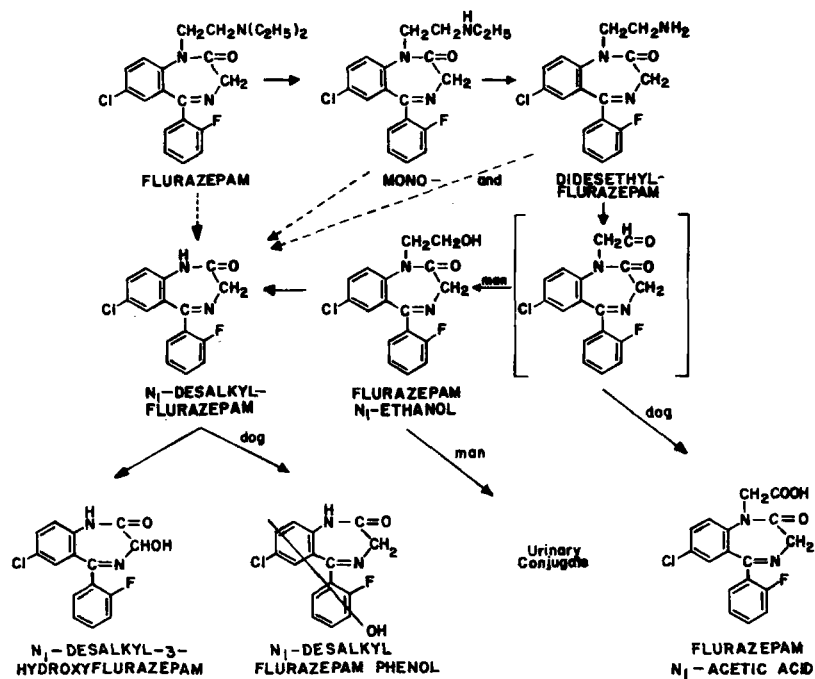
#### 6.21 Organically Bound Fluorine Analysis

There are several methods available to determine the amount of carbon-bonded fluorine. One of the earlier methods employed the Schöniger Combustion technique followed by thorium nitrate or cerous chloride titration using sodium alizarin sulfonate or murexide as the indicator (24).

With the advent of good specific ion

Figure 9

Metabolic Products of Flurazepam Hydrochloride



electrodes, methods were developed to liberate the bound fluorine and directly measure the fluoride concentration. The reagent, sodium-biphenyl, followed by oxidation with hydrogen peroxide, is used to liberate the organically bound fluorine in flurazepam hydrochloride. A fluoride ion specific electrode is used, in the presence of a high-ionic-strength buffer solution, for direct measurement of the liberated fluoride (25).

The last method to be presented for the analysis of the carbon-bonded fluorine in flurazepam hydrochloride is  $^{19}\text{F}$  Nuclear Magnetic Resonance spectrometry (2). A flurazepam hydrochloride reference standard and an internal standard, reagent grade o-fluorobenzoic acid, are dissolved in methanol and the  $^{19}\text{F}$  spectrum obtained and integrated. From this data an internal standard fluorine conversion factor can be calculated and used to determine the amount of fluorine present in a sample of flurazepam hydrochloride that is run in a similar manner (26).

#### 6.22 Free Fluoride Analysis

The determination of any free fluoride present in flurazepam hydrochloride bulk samples is carried out by direct measurement using a fluoride specific ion electrode. The measurements are made in an acetate buffer solution ( $\text{pH} \approx 5.3$ ). The electrode response was found to be linear throughout the working range of 0.08 to 0.20 mg of  $\text{F}^-$ /100 ml of solution (27).

#### 6.3 Thin Layer Chromatographic Analysis (TLC)

Several TLC systems for the separation of flurazepam hydrochloride from its metabolites and similar structured compounds are given in Table VI. In each case the sample is spotted on a silica gel GF plate\* which is allowed to develop in a saturated tank until the solvent front has ascended about 15 cm. The plate is then removed, air dried, and viewed under shortwave and longwave U.V. radiation.

\* If the sample solution is too acidic, an artifact appears at the point of application due to the quenching of the phosphor in the silica gel GF plate by the acid.

## FLURAZEPAM HYDROCHLORIDE

Table VI

R<sub>f</sub> Values for Flurazepam in Various Developing Solvents

<u>Solvent System</u>	<u>R<sub>f</sub> Value</u>	<u>Reference</u>
diethyl ether:diethylamine (75:2)	0.6	28
methylene chloride:ethyl ether: methanol:conc. ammonium hydroxide (240:360:8:3)	0.2	28
ethyl acetate:conc. ammonium hydroxide (200:1)	0.14	20
ethyl acetate:ethanol:conc. ammonium hydroxide (100:10:0.3)	0.38	20
benzene:methanol:glacial acetic acid (9:1:1)	0.26	20
chloroform:acetone (17:3)	0.00, 0.05*	20

\* When the plate is developed two times in the same system.

6.4 Gas-Liquid Chromatographic Analysis (GLC)

The acid hydrolysis of blood extracts containing flurazepam and its metabolites has been used by deSilva et al. (29) to prepare the respective benzophenones as volatile derivatives for gas chromatography. This method is an adaptation of the method developed for GLC of diazepam and its metabolites (30). When the benzophenones were chromatographed at 210°C on a 2 feet x 1/4 inch column containing 2% Carbowax 20M-TPA, they showed an excellent response to detection by electron capture which was linear between 10 and 40 ng. The main disadvantage of hydrolysis to the benzophenone is the lack of specificity for a given benzodiazepine.

A method recently published by Sine et al. (31) for chromatographing flurazepam directly, utilizes a 3 feet x 2 mm glass column packed with 3.8% SE-30 on Chromosorb W (AW-DMCS, 80-100 mesh). The GLC is equipped with a hydrogen flame ionization detector and the column temperature is about 230°C. The patient's serum is adjusted to pH 7.4 and extracted with chloroform. The chloroform is evaporated, the residue is dissolved in acidic methanol (1 ml HCl/liter methanol) and chromatographed.

This method will separate flurazepam from diazepam and chlordiazepoxide.

#### 6.5 Polarographic Analysis

Polarographic analysis of flurazepam hydrochloride has been carried out in Britton-Robinson Buffer at pH 4.4. The halfwave potential occurs at -0.78 V. versus a silver/silver chloride reference electrode and is proportional to concentration. This wave is attributed to the reduction of the azomethine ( $\text{C}=\text{N}$ ) functional group and varies with pH (32).

#### 6.6 Direct Spectrophotometric Analysis

Direct spectrophotometric analysis is used to determine the quantity of flurazepam hydrochloride present in capsules. A quantity of the capsule contents is accurately weighed and the flurazepam is extracted into acidified methanol (see section 2.3). The methanol solution is filtered and appropriate subdivisions made to yield a final solution containing 1.0 mg of flurazepam hydrochloride per 100 ml of acidified methanol. The absorbance of this solution along with a solution of flurazepam hydrochloride reference standard similarly prepared is measured versus acidified methanol at the 239 nm maximum. From this data the concentration of flurazepam hydrochloride in the capsules is calculated (33).

#### 6.7 Colorimetric Analysis

Flurazepam hydrochloride forms a ion-pair complex with bromocresol green in a pH 5.3 buffer. This colored complex is extracted into chloroform and its absorbance measured at the 415 nm maximum. A plot of concentration versus absorbance is linear from 0 to 2.5 mg of flurazepam hydrochloric per 100 ml of chloroform (34).

#### 6.8 Fluorimetric Analysis

A fluorimetric analysis for the determination of flurazepam hydrochloride and its metabolites in blood and urine has been described by de Silva and Strojny (20). This assay involves selective extraction into diethyl ether from blood buffered to pH 9 or urine made basic with NaOH, then back-extracted into 4N HCl, and hydrolyzed to the respective benzophenones. The benzophenones are then cyclized to the 9-acridone derivatives in dimethylformamide

## FLURAZEPAM HYDROCHLORIDE

in the presence of  $K_2CO_3$ . These derivatives are separated by TLC, eluted from the silica gel, and their fluorescence determined in methanol:0.1N HCl (4:1). This method allows quantitation in the range of 0.003 to 10.0 mcg of compound/ml of blood or urine (20).

### 6.9 Titrimetric Analysis

Flurazepam hydrochloride is assayed by dissolving about 0.6 gm of sample in glacial acetic acid, adding excess mercuric acetate, and titrating with 0.1N perchloric acid in glacial acetic acid. The end-point is determined potentiometrically using a glass-calomel electrode system. Each ml of 0.1N perchloric acid is equivalent to 23.04 mg of  $C_{21}H_{23}ClFN_3O \cdot 2HCl$ .

### 7. Acknowledgment

The authors would like to thank Dr. P. Sorter and the Scientific Literature Department as well as the Research Records Department of Hoffmann-La Roche Inc. for their help in the literature search for this Analytical Profile.



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## **IODIPAMIDE**

*Hyam Henry Lerner*

## Table of Contents

1. Description
  - 1.1 Name, Formula, Molecular Weight
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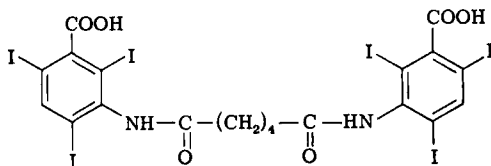
## 1. Description

### 1.1 Name, Formula, Molecular Weight

Iodipamide is N,N'-adipyl bis (3-amino-2,4,6 triiodobenzoic acid). Chemical Abstract listings are under the heading benzoic acid, 3,3' (adipyl-diimino) bis [2,4,6 triiodo]. Other derived chemical names are adipic acid di-(3-carboxy-2,4,6 triiodoanilide; N,N'-di-(3-carboxy-2,4,6-triiodophenyl)-adipamide and 3,3'-(adipoyldiimino)-bis [2,4,6-triiodobenzoic acid].

Among the generic and trivial names for this compound are iodipamic acid and adipiodon. Common trade names are Biligrafin and Cholografin.

Iodipamide was officially recognized in "National Formulary XI." United States Pharmacopoeia XVIII continues this name in a monograph for Meglumine Iodipamide Injection.



$C_{20}H_{14}I_6N_2O_6$

Mol. Wt. 1,139.7

### 1.2 Appearance, Color, Odor

Iodipamide is a white, odorless and tasteless crystalline powder<sup>1,11,12</sup>. The disodium salt has a sweet, metallic taste followed by a bitter aftertaste<sup>12</sup>.

## 2. Physical Properties

### 2.1 Spectra

#### 2.11 Infrared Spectra

The spectra of iodipamide in Figures 1a and 1b were determined on a Perkin-Elmer Model 621 grating infrared spectrophotometer. Samples of iodipamide were dispersed in a potassium bromide pellet or in mineral oil<sup>49</sup>.

The following spectral assignments were made by Toeplitz<sup>50</sup> on the spectrum obtained from the sample dispersed in mineral oil (Figure 1b):

<u>cm<sup>-1</sup></u>	<u>Assignment</u>
3200	N-H
2500, 1900	N-H and OH of amido acid
1690	C=O of carboxyl group
1610	C=O of amide
1530	secondary amide
1280	C-OH of carboxyl group

The spectra shown in Figures 1a and 1b are dissimilar. An explanation was advanced by Toeplitz<sup>50</sup>, who suggested that iodipamide might be reacting with potassium bromide.

Herrmann<sup>1</sup> published an infrared spectrum obtained on a potassium bromide dispersion that agrees qualitatively with the spectrum in Figure 1a. Neudert and Röpke<sup>3</sup> published an infrared spectrum that does not agree with the spectrum in either Figure 1a or Figure 1b.

#### 2.12 Nuclear (Proton) Magnetic Resonance Spectrum

The NMR spectrum of iodipamide in Figure 2 was determined on a Varian XL-100 NMR spectrometer<sup>2</sup> at ambient probe temperature (ca. 31°). The sample was dissolved in deuterated dimethylsulfoxide containing tetramethylsilane as an internal reference ( $\text{Me}_4\text{Si} = 0$  ppm). Spectral assignments of the peaks are recorded in Table I.

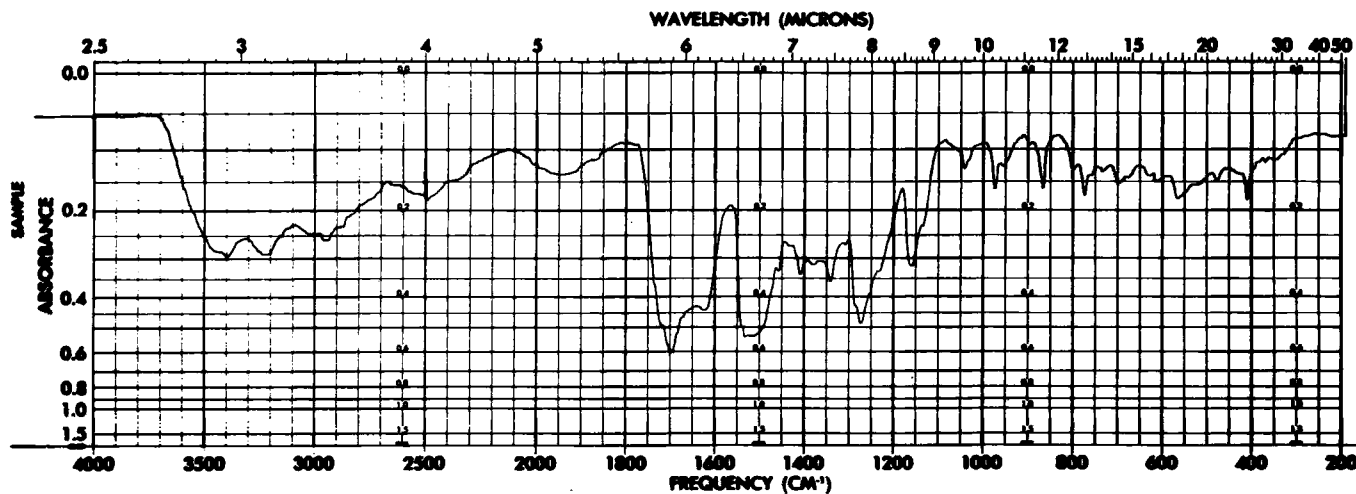


Figure 1a. Infrared Spectrum of Iodipamide, Squibb Lot 03122, from KBr pellet.  
Instrument: PE Model 621 Infrared Spectrophotometer



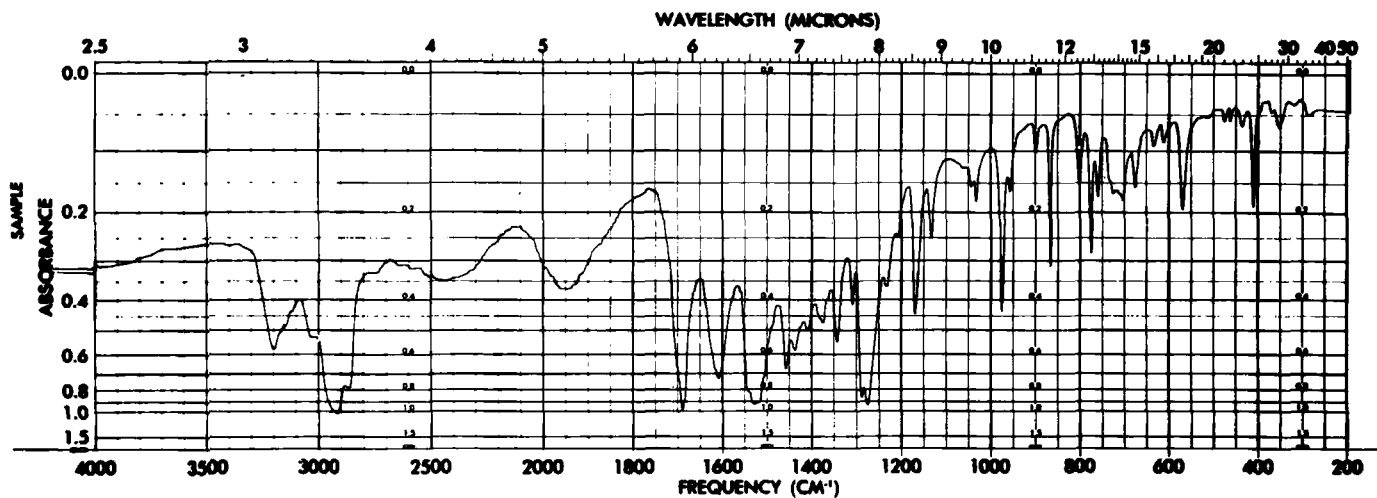


Figure 1b. Infrared Spectrum of Iodipamide, Squibb Lot 03122, from mineral oil mull.  
Instrument: PE Model 621 Infrared Spectrophotometer

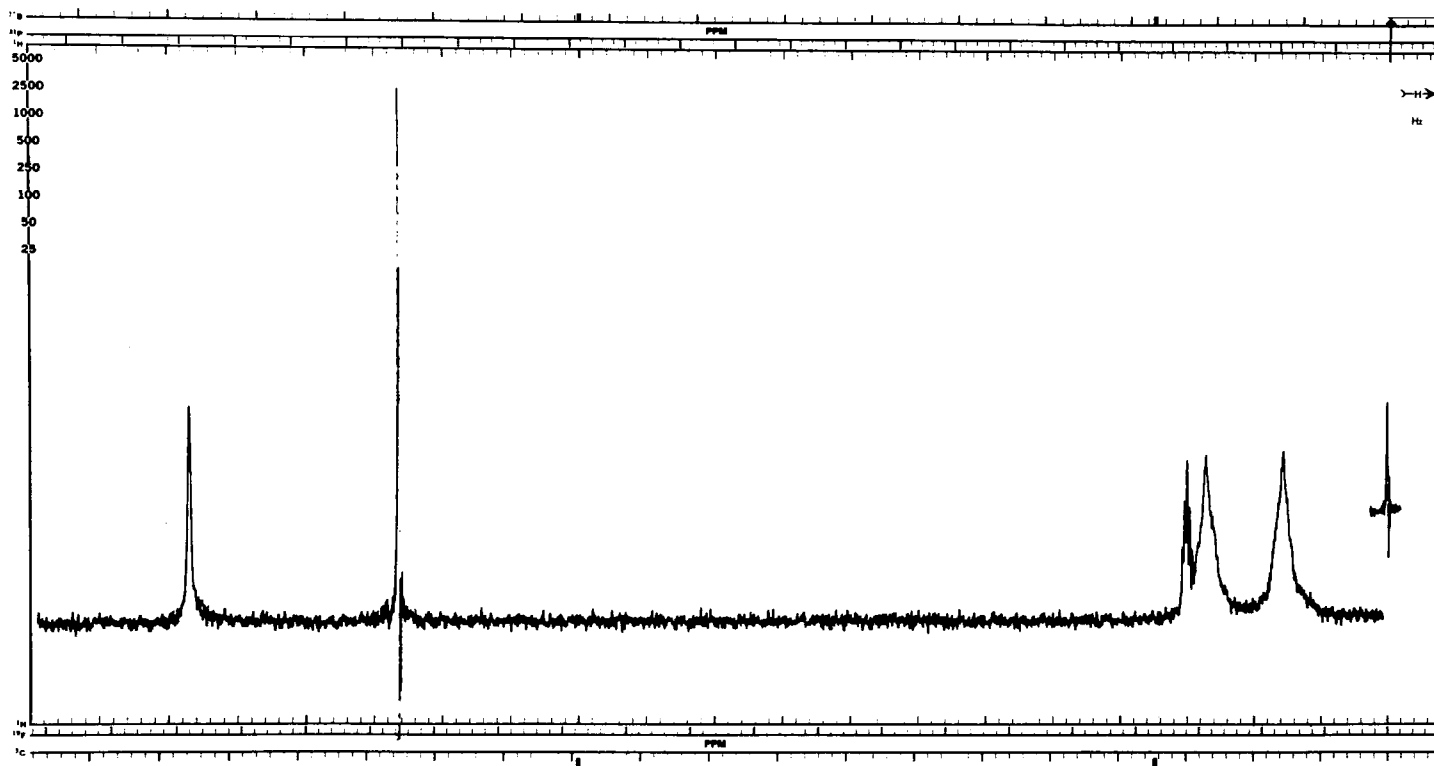


Figure 2. NMR Spectrum of Iodipamide, Squibb Lot 03122 in DMSO-d<sub>6</sub>.  
Instrument: Varian-XL100 NMR Spectrometer

In deuterated water and deuterated sodium hydroxide, the peak at  $\delta 9.86$  was absent, indicating exchange of the amine proton. The carboxylic acid protons were not located, probably because of hydrogen bonding. High-field methylene resonance indicated the absence of other groups attached to the methylene groups.

Table I  
NMR Spectral Assignments

<u>Assignment</u>	<u>Chemical Shift (ppm, <math>\delta</math>)</u>	<u>No. of Protons</u>
-CH <sub>2</sub> -CH <sub>2</sub> -   O	1.78 (s)	4
-C-CH <sub>2</sub> -	2.36 (m)	4
aromatic	8.33 (s)	2
-NH	9.86 (s)	2
-COOH	not located	2

s = singlet; m = multiplet

### 2.13 Ultraviolet Spectra

The following ultraviolet spectral data have been reported for iodipamide:

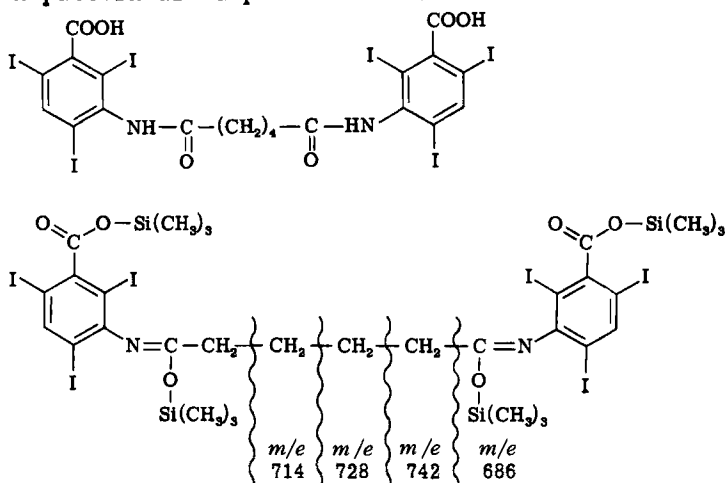
<u>Solvent</u>	<u><math>\lambda_{\max}</math>, nm</u>	<u><math>\epsilon</math></u>	<u>Reference</u>
0.01N NaOH	238	70,700	48
0.1N NaOH	236	72,000	48
0.1N NaOH	236	71,800	1
0.1N KOH	237	72,400	4
Methanol	238	68,000	8
Methanol	239	71,800	4
0.15M NaCl	238	73,200	5
0.15M Phosphate Buffer (pH 5.8)	238	73,200	5

Neudert and Röpke<sup>3</sup> reported the  $\epsilon$  value of the disodium salt in methanol, at the 239 nm maximum, to be 74,700. Ostrow and Levy<sup>5</sup> reported their data in terms of absorbance per micromole of iodine. Sodium iodide, which peaks at 226 nm, has the same absorbance per micromole of

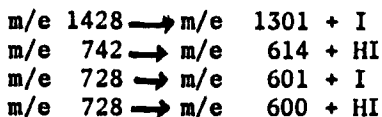
iodine as does iodipamide, which suggests that the ultra-violet absorption of iodipamide results from the presence of iodine chromophores.

## 2.14 Mass Spectrometry

No molecular ion is observed for iodipamide because of its low volatility and because of the thermal degradation of the compound. Per-trimethyl silylation by Funke<sup>6</sup> yielded a compound with a molecular ion of  $m/e$  1428, consistent with the replacement of four protons by four trimethyl silyl groups. The structure and major fragmentation pattern are depicted below:



Other fragments that have been found are due to loss of I or HI and include:



## 2.2 Crystal Properties

### 2.21 Differential Thermal Analysis

Valenti<sup>7</sup> determined the DTA of iodipamide on a Du Pont 900 Thermoanalyzer at a temperature rise of 15° per min. A single endotherm at 308° and a single

exotherm at 314° were detected. The thermogram is reproduced in Figure 3.

## 2.22 Thermal Gravimetric Analysis

Valenti<sup>7</sup> determined the TGA of iodipamide on a Du Pont Thermogravimetric Analyzer. When the compound was heated at a rate of 15° per minute under nitrogen sweep, no weight loss was observed below 250°.

## 2.23 Melting Range

Willis<sup>48</sup> reported a melting range for iodipamide of 306.5 - 308°, with decomposition, as determined on a Thomas-Hoover Capillary Melting Point apparatus. Priewe and Rutkowski<sup>13</sup> reported the melting range to be 306° - 308°, with decomposition. Herrmann<sup>1</sup> reported that the compound decomposes above 280°.

Hoevel-Kestermann and Muhlemann<sup>9</sup> determined the melting range on a Kofler Microblock (Reichert) and reported the melting range to be 289 - 290°, with decomposition. This latter value appears to be in error, when compared with the previously cited DTA data (Section 2.21) and measurements made with the capillary melting point apparatus.

## 2.24 X-Ray Powder Diffraction

Ochs<sup>10</sup> obtained the X-ray powder diffraction spectrum of iodipamide on a Phillips X-Ray Powder Diffractometer, at a voltage of 45 kv and a current of 15 ma. The sample was irradiated by a copper source at 1.54A. Diffraction data for Squibb Lot 03122 are recorded in Table II.



Figure 3. DTA Thermogram of Iodipamide, Squibb Lot 03122.  
Instrument: Du Pont 900 Thermoanalyzer

\* SEE INSTRUCTION MANUAL FOR SCALE CORRECTION

IODIPAMIDE

Table II

X-Ray Powder Diffraction Pattern of Iodipamide,  
Squibb Lot 03122

<u>d (Å)*</u>	<u>Relative Intensity**</u>
8.80	0.25
7.40	0.18
5.70	0.13
5.5	0.19
5.10	0.19
4.46	0.34
4.40	0.60
4.29	0.51
4.23	0.39
4.16	0.34
4.09	1.00
3.93	0.69
3.80	0.15
3.67	0.25
3.64	0.18
3.47	0.16
3.44	0.16
3.37	0.10
3.30	0.15
3.25	0.56
3.17	0.28
3.14	0.39
3.10	0.40
2.99	0.29
2.96	0.43
2.92	0.20
2.90	0.20
2.84	0.14
2.66	0.22
2.61	0.17
2.56	0.25
2.51	0.17
2.43	0.13
2.32	0.13

\*d = (interplanar distance)  $\frac{n\lambda}{2 \sin \theta}$

where  $\lambda = 1.539\text{Å}$

\*\* Based on highest intensity of 1.00

2.3 Solution Data2.31 Solubility

The following data were reported for the solubility of free acid of iodipamide at room temperature:

<u>Solvent</u>	<u>Solubility (mg/100 ml)</u>			
	<u>Ref.<sup>9</sup></u>	<u>Ref.<sup>12</sup></u>	<u>Ref.<sup>8</sup> at 20°</u>	<u>Ref.<sup>7</sup></u>
Acetone	-	200	-	<20
Ethanol, 95%	230	-	-	<20
Ether	30	100	-	<20
Chloroform	0.1	-	-	<20
Methanol	758	800	440	-
Water	16	insoluble	46	<20
0.1N sodium hydroxide	-	-	-	5,240
n-hexane	-	-	-	<20
Benzene	-	insoluble	-	<20
Propylene glycol	-	-	-	<20
0.1N hydrochloric acid	-	-	-	<20
Ethylene glycol	-	300	-	-
Tetrahydrofuran	-	-	1,200	-
Tetrahydrofurfuryl alcohol	-	-	8,200	-

Neudert and Röpke<sup>8</sup> also reported the solubility of iodipamide in acetamide, urethan and phenol, at the melting point of the solvents, to be 3 g, 0.5 g, and 0.3 g per 100 g of solvent, respectively.

The solubility at 20° of the disodium and dilithium salts of iodipamide were reported<sup>8</sup>:



<u>Solvent</u>	<u>Solubility (g/100 ml)</u>	
	<u>Disodium Salt</u>	<u>Dilithium Salt</u>
Water	350	450
Methanol	14	65
Tetrahydrofuran	4	3
Tetrahydrofurfuryl alcohol	4	-

### 2.32 Apparent Molecular Weight in Solution

According to Neudert and Röpke<sup>8</sup>, iodipamide forms micelles and has soap-like properties. Colloidal solutions contain molecular aggregates with an apparent molecular weight 16 times that of the empirical formula of iodipamide.

### 2.33 Isotonicity

A 14.6% (w/v) solution of the disodium salt of iodipamide (0.1233M) is isotonic with physiological salt solution<sup>12,24</sup>.

### 2.34 pKa

The pKa of the free acid of iodipamide was reported<sup>8,24</sup> to be 3.5. This value may be a composite of pKa<sub>1</sub> and pKa<sub>2</sub>, since both dissociation constants can be expected to be similar in value.

### 2.35 pH

The pH of a 1% suspension of iodipamide was reported<sup>9</sup> to be 3.95. Herrmann<sup>1</sup> proposed limits for a saturated solution of 3.5 - 3.9.

Iodipamide neutralized with sodium hydroxide was reported<sup>8</sup> to have a pH of 7.4.

### 2.36 Index of Refraction

The refractive index of iodipamide at 21.5°, in methanol<sup>12</sup>, is given in Table III. Neudert and Röpke<sup>8</sup> reported the refractive index of the disodium salt, at a

concentration of 35 g/100 in water, and measured with the D-line of sodium, to be 1.4016.

Table III

Refractive Index of Iodipamide at 21.5°C in Methanol<sup>12</sup>

<u>g/100 ml</u>	<u>n<sub>D</sub></u>
0.0	1.3278
0.116	1.3283
0.263	1.3288
0.348	1.3292
0.445	1.3294

### 2.37 Physicochemical Data

The freezing-point depression ( $-\Delta T$ ), degree of dissociation ( $\alpha$ ), and osmotic pressure ( $P_o$ ) of the disodium salt of iodipamide in aqueous solution were reported<sup>8,12</sup>, and are recorded in Table IV.

Table IV

Physicochemical Data<sup>8,12</sup> for the Disodium Salt of Iodipamide:

Freezing-Point Depression ( $-\Delta T$ ), Degree of Dissociation ( $\alpha$ ), and Osmotic Pressure ( $P_o$ )

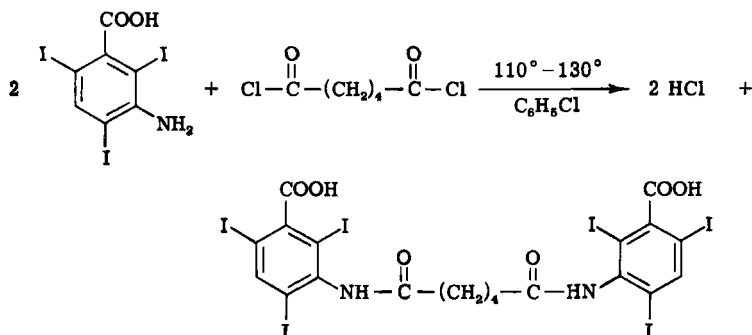
<u>g/100 ml</u>	<u>Molarity</u>	<u><math>-\Delta T</math></u>	<u><math>\alpha</math></u>	<u><math>P_o</math></u>
2.0	0.0170	0.095	1.00	1.13
5.0	0.0422	0.226	0.94	2.69
10.0	0.0844	0.425	0.85	5.04
14.6	0.1233	0.557	0.72	6.64
21.6	0.1825	0.720	0.56	8.56

### 3. Synthesis

Iodipamide is prepared by the reaction of 2,4,6-triiodo-3 aminobenzoic acid with adipic acid dichloride<sup>13</sup>. The former is dissolved in chlorobenzene and heated to 110 - 130°C. The adipic acid dichloride is added dropwise,

resulting in the evolution of hydrochloric acid. When the evolution of HCl has ceased, the precipitated crude product is filtered, with suction, while still hot. The crude precipitate is washed with chlorobenzene, and the residual chlorobenzene is extracted by boiling with methanol. The precipitate is dissolved in caustic methanol, filtered through charcoal, and precipitated with dilute hydrochloric acid.

An alternate solvent for the reaction of 2,4,6-tri-iodo-3 aminobenzoic acid with adipic acid dichloride is toluene<sup>14</sup>.



#### 4. Stability

Iodipamide is chemically stable at room temperature. By extrapolation to room temperature of the data for activation energy and frequency constant of the reaction between 70 and 110°, the decomposition at the N-acyl bond was calculated to be 0.1% in 50 yr.<sup>33</sup> Evolution of iodine at 90° from very pure iodipamide in solution at pH 9 is less than 3% in 75 hr. The presence of impurities such as under-iodinated compounds, enhances decomposition<sup>33</sup>.

Formulated neutralized (pH 7.2) solutions containing iodipamide (192 mg/ml), sodium citrate (3.2 mg/ml), and sodium edetate (0.28 mg/ml), show little or no loss of potency after storage for 5 yr. at room temperature<sup>15</sup>.

#### 5. Purification and Analysis for Impurities

Purification procedures used for analysis are described under Chromatographic Analysis (Section 6.6).

### 5.1 Gel Filtration

Ostrow and Levy<sup>5</sup> attempted to isolate and purify iodipamide on 1.0- x 12.5-cm columns prepared from dextran gel (Sephadex G-10 and G-25, Pharmacia) and polyacrylamide gel (Bio-Gel P-2, Bio-Rad). Samples of 0.2 ml, containing 0.3 to 8.6  $\mu$ moles of iodipamide, were eluted with 0.15M sodium phosphate buffer (pH 5.8). The eluted fractions were measured spectrophotometrically at 226, 238, and 255 nm. Results are shown in Table V. Due to adsorption of iodipamide on the gel, recoveries were not quantitative. Contaminants closely related to iodipamide were shown, by paper chromatography of the iodipamide fractions, to be present even after chromatography on Sephadex Gel G-10.

Table V

#### Gel Filtration of Iodipamide

##### Profile on Three Different Gels, with pH 5.8 Phosphate Buffer as Elution Solvent<sup>5</sup>

<u>Component</u>	<u>Sephadex Gel G-25</u>	<u>Sephadex Gel G-10</u>	<u>Bio Gel P-2</u>
Iodipamide	8-32 ml (peak at 20 ml)	eluted at void volume - tail- ing till 50 ml	8-50 ml (peak at 16 ml)
Sodium Iodide	10-24 ml (peak at 15 ml)	28-50 ml (peak at 37 ml)	11-25 ml (peak at 18 ml)

### 5.2 Complexometric Methods of Separation

Hentrich and Pfeifer<sup>34</sup> described methods for the precipitation of ten contrast agents as the metallic salts or metallic complex salts. Iodipamic acid can be precipitated quantitatively by silver nitrate, cadmium sulfate with thiourea, cadmium sulfate with pyridine, copper sulfate with thiourea, and copper sulfate with pyridine. Chelatometric methods are also described for the titration of excess precipitant, after separation of the precipitated salt by filtration. The formulas of the precipitated salts and complexes, their molecular weights, melting ranges, and

the equivalent weight of the iodipamide precipitated by 1 ml of 0.1M solution of the inorganic precipitant are given in Table VI.

Table VI  
Salt Complexes of Iodipamic Acid<sup>34</sup>

<u>Precip- itant</u>	<u>Mol. Formula of Salt</u>	<u>M.W. of Salt</u>	<u>Melting Range</u>	<u>Equiv. Weight to 1 ml of 0.1M Precipitant</u>
AgNO <sub>3</sub>	C <sub>20</sub> H <sub>12</sub> I <sub>6</sub> N <sub>2</sub> O <sub>6</sub> Ag <sub>2</sub>	1,353.6	212°-214°	0.0570 g
CdSO <sub>4</sub> - Thiourea	Cd[(NH <sub>2</sub> ) <sub>2</sub> CS] <sub>4</sub> · C <sub>20</sub> H <sub>12</sub> I <sub>6</sub> N <sub>2</sub> O <sub>6</sub>	1,554.7	237°-238°	0.1140 g
CdSO <sub>4</sub> - Pyridine	Cd[(C <sub>5</sub> H <sub>5</sub> N) <sub>4</sub> ] <sub>2</sub> · C <sub>20</sub> H <sub>12</sub> I <sub>6</sub> N <sub>2</sub> O <sub>6</sub>	1,586.6	290°	0.1140 g
CuSO <sub>4</sub> - Thiourea	{Cu[(NH <sub>2</sub> ) <sub>2</sub> C- S] <sub>2</sub> } <sub>2</sub> ·C <sub>20</sub> H <sub>12</sub> - I <sub>6</sub> N <sub>2</sub> O <sub>6</sub>	1,569.4	167°	0.0570 g
CuSO <sub>4</sub> - Pyridine	[Cu(C <sub>5</sub> H <sub>5</sub> N) <sub>2</sub> ] <sub>2</sub> · C <sub>20</sub> H <sub>12</sub> I <sub>6</sub> N <sub>2</sub> O <sub>6</sub>	1,359.5	202°-203°	0.1140 g

### 5.3 Countercurrent Distribution

Strickler et al<sup>20</sup> separated iodipamide and other contrast agents from sera by countercurrent distribution. They used a solvent system composed of sec-butanol: dilute aqueous ammonia (1:1). Both a 30-tube manual procedure and a 200-tube automatic procedure are described.

### 5.4 Free Iodine and Free Halide

Free iodine can be detected by boiling iodipamide with water for 2 min, filtering, and observing a blue color after treatment with starch. After acidification of another portion of filtrate with dilute nitric acid and treatment of it with silver nitrate test solution, the presence of free halide ions can be detected by the

occurrence of opalescence or turbidity<sup>1,27</sup>.

Hartmann and Röpke<sup>33</sup> quantitated free iodide by titrating potentiometrically with 0.001 N silver nitrate, under a protective cover of nitrogen.

### 5.5 Free Amino Compounds

Hartmann and Röpke<sup>33</sup> determined iodinated impurities having a free amino group, by a kinetic method based on the more rapid reactivity of these impurities with elemental bromine in acetic acid solution than is shown by iodipamide. Under these conditions, the free amino compounds quantitatively split off iodine, which is then oxidized to iodate by the bromine. After destruction of the excess bromine, the iodate is reduced to iodine and titrated with sodium thiosulfate, to a starch endpoint. Free-iodide compounds also react with bromine and would give positive results by this method.

Hoevel-Kestermann and Muhlemann<sup>9</sup> described a Bratton-Marshall colorimetric reaction for the detection of free amino groups. Hartmann and Röpke<sup>33</sup> used the Bratton-Marshall reaction to quantitate free amino compound impurities.

### 5.6 Free Adipic Acid

Hoevel-Kestermann and Muhlemann<sup>9</sup> described a thin-layer chromatographic procedure for adipic acid, after its cleavage from iodipamic acid. This procedure is described in Section 6.2 and can be adapted to determine free adipic acid.

### 5.7 Determination of Water and Conditions for Drying

Herrmann<sup>1</sup> dried iodipamide at 105° for 4 hr. Hoevel-Kestermann and Muhlemann<sup>9</sup> dried iodipamide over phosphorus pentoxide for 24 hr. In the "National Formulary XI," water is determined by the Karl Fischer titrimetric method.

## 6. Methods of Analysis

### 6.1 Elemental Analysis

<u>Element</u>	<u>% Theory</u>	<u>% Reported<sup>25</sup></u>
C	21.075	21.16
H	1.238	1.35
I	66.806	67.28
N	2.458	2.30
O	8.422	-

### 6.2 Identification Tests

Infrared (Section 2.11), paper chromatography (Section 6.61), and thin-layer chromatography (Section 6.62) have been used to identify iodipamide.

Shamotienko<sup>45</sup> identified iodipamide by boiling it with trichloroacetic acid and a 5% aqueous solution of chloramine. Iodipamide gives a cloudy yellow solution with a yellow precipitate.

The evolution of intense violet fumes of liberated iodine can be observed by heating a sample of iodipamide over an open flame<sup>1,9,11</sup>.

Identification of the bound amine group can be made by first ascertaining the absence of free amino groups (Section 5.6) and then cleaving the molecule by refluxing in base and repeating the Bratton-Marshall reaction<sup>9</sup>.

Iodipamide may be hydrolyzed by refluxing with hydroiodic acid to liberate adipic acid. The adipic acid can be extracted with ether and chromatographed on silica gel G plates. The solvent system benzene:dioxane:acetic acid (65:25:29) was used. Adipic acid ( $R_f$  0.64) can be detected visually by spraying the developed plate with bromocresol green<sup>9</sup>.

### 6.3 Direct Spectrophotometric Analysis

The strong ultraviolet band of iodipamide at 238  $\pm$  2 nm in basic solution and methanol (Section 2.13) can be used for direct spectrophotometric analyses<sup>4</sup>. The

absorption band is said to result from the iodine chromophores<sup>5</sup>. Detection and quantitation of eluates from chromatographic separations are easily accomplished by using this band for analyses.

#### 6.4 Organically Bound Iodine

Under reflux conditions, the iodine in iodipamide can be reduced and replaced by hydrogen, generated by the reaction of powdered zinc and sodium hydroxide. The iodide is determined by titration with standardized silver nitrate in acid solution in the presence of tetrabromophenolphthalein ethyl ester indicator solution<sup>1,11</sup>.

Ates and Amal<sup>26</sup> decomposed iodipamide with alkaline permanganate<sup>27,28</sup>. After decoloration of the permanganate with sodium nitrite and acid, they titrated the liberated iodine with 0.1N sodium thiosulfate.

Yakatan and Tuckerman<sup>29</sup> reviewed four methods for decomposing contrast agents to liberate organically bound iodine: A. Parr bomb (fusion with sodium peroxide); B. alkaline permanganate reduction; C. zinc-sodium hydroxide reduction; and D. oxygen flask (Schöniger) combustion. Method D is recommended as a general technique for all iodinated organic compounds because of its reproducibility, simplicity, and rapidity. For compounds that have all the iodine atoms ortho or para to the electronegative carboxylic acid on the aromatic ring, e.g., iodipamide, Method C (zinc-sodium hydroxide reduction)<sup>1,11</sup> is recommended.

Krasnova<sup>30</sup> recommended a modification of the oxygen-flask combustion method of Yakatan and Tuckermann<sup>29</sup>.

Hoewel-Kestermann and Muhlemann<sup>9</sup> reviewed three methods for liberating organically bound iodine in contrast agents: A. Parr bomb (fusion with sodium peroxide); B. catalytic reduction with sodium borohydride; and C. zinc-sodium hydroxide reduction. They recommend the sodium borohydride reduction because of its simplicity and short assay time. The sodium borohydride reduction was originally proposed by Egli<sup>31</sup>.



## 6.5 Polarography

Vaskelis *et al.*<sup>32</sup> determined contrast agents polarographically in 0.1N potassium chloride containing gelatin. Iodipamide yields a single wave, with a half-wave potential of -1.2v vs SCE. Results were quantitated from prepared calibration curves.

## 6.6 Chromatographic Analysis

### 6.61 Paper Chromatography

Many descending paper chromatographic methods have been found suitable for the isolation and detection of iodipamide. These are summarized in Table VII. Some of the references cited give sample preparation techniques and methods of eluting the drug from the developed chromatogram to permit quantitation by ultraviolet spectrophotometry (Section 2.13) or other means. Pileggi *et al.*<sup>22</sup> described a method for separating 19 organic iodide compounds from blood serum.

Table VII

Paper Chromatographic Systems for Iodipamide

<u>Solvent System</u>	<u>Paper</u>	<u>Method of Detection</u>	<u>Reference</u>
I	Whatman No. 1	A	35
II	Whatman No. 4	A	36
III	Whatman No. 1	B	5
IV	Whatman No. 3	B,C	26,39
V	Whatman No. 3	B,C	26,39
VI	Whatman No. 3	D	22

### Solvent Systems

- I - n-butanol:1N ammonium hydroxide:ethanol (5:2:1)
- II - H<sub>2</sub>O:n-butanol:ethanol (5:4:1); upper phase used for development, lower phase to equilibrate chamber.

Table VII (Cont'd)

- III - n-butanol:0.5N ammonium hydroxide:ethanol:H<sub>2</sub>O (20:20:2:1); upper phase used for development, lower phase + 20 ml of upper phase used to equilibrate chamber.
- IV - Ethanol:25% ammonia (ratio of solvents not given)
- V - Methanol:2N acetic acid (ratio of solvents not given)
- VI - sec-butanol:ammonia 4% (3:1)

Methods of Detection

- A. Long-wave ultraviolet light.
- B. Spray with 10% ceric sulfate and 5% sodium arsenite, both prepared in 1N sulfuric acid<sup>37</sup>.
- C. Short-wave ultraviolet light.
- D. Spray with mixture of ceric ammonium sulfate and arsenious acid, followed by spraying with 0.5% solution of methylene blue<sup>42</sup>.

6.62 Thin-Layer Chromatography

Thin-layer chromatographic methods found suitable for the separation and detection of iodipamide are summarized in Table VIII. Some of the references cited present sample preparation techniques and methods for eluting the drug from the developed plate to permit quantitation by ultraviolet spectrophotometry (Section 2.13) or other means.

Hollingsworth et al.<sup>41</sup> separated iodoamino acids and related compounds on cellulose plates, with a solvent system composed of tert.-butanol:2N ammonia:chloroform (376:70:60). They did not report using this technique on iodipamide. However, it seems reasonable to expect that this system will separate iodinated contrast agents. Stahl and Pfeifle<sup>38</sup> reported on six systems used to separate 17 iodinated compounds and Hoevel-Kestermann and Muhlemann<sup>9</sup> separated 8 contrast agents with one system.

Table VIIIThin-Layer Systems for Separation of Iodipamide

<u>Solvent System</u>	<u>Plate</u>	<u>Detection System</u>	<u>R<sub>f</sub></u>	<u>Reference</u>
I	A	a,b	0.09	9
II	B,C	a,c	not given	26,39
III	B,C	a,c	not given	26,39
IV	A	a,b	0.27	38
V	A	a,b	0.33	38
VI	A	a,b	0.33	38
VII	D	a	0.20	40

Solvent Systems

- I - Ethyl acetate:isopropyl alcohol:ammonia 25% (11:7:4)
- II - Methanol:ammonia, 25% (2:1)
- III - Methanol:2N acetic acid (10:1)
- IV - Acetone:isopropyl alcohol:ammonia, 25% (2:2:1)
- V - Isopropyl alcohol:ammonia, 25% (4:1)
- VI - Ethylacetate:methanol:diethylamine (5:4:2)
- VII - n-butanol:ethanol:1N ammonia (5:1:2)

Plate

- A. 30 g of silica gel HF<sub>254</sub>, 70 ml of H<sub>2</sub>O and 0.5 g of starch.
- B. Silica Gel G (Merck).
- C. Silica Gel H<sub>254-366</sub> (Merck).
- D. Eastman "Chromogram" #6061 silica gel, plastic plates.

Table VIII (Cont'd)Detection System

- A. Short-wave ultraviolet light (254 nm).
- B. Spray with 50% solution of acetic acid, followed by irradiation at 254 nm for 10 min to give blue-violet spots. Spraying and irradiation may be repeated to increase intensity of the spots.
- C. Spray with 1:1 solution of 10% ceric sulfate and 5% sodium arsenite, both in 1N sulfuric acid<sup>37</sup>.

6.63 Electrophoretic Analysis

Ardoino and Pavone<sup>46</sup> reported on the electrophoretic analysis of contrast agents in biological secretions from the liver and kidney. Iodipamic acid migrates at the speed of albumin and with albumin.

6.7 X-Ray and  $\beta$ -Particle Dispersion Methods

Holynska and Jankiewicz<sup>43</sup> used X-ray fluorescence and absorption techniques to determine iodine in various contrast agents. In the X-ray fluorescence work, excitation was obtained by irradiation of the sample, from a <sup>241</sup>Am source of 5 mCi activity. Energy of excitation was 60 keV. The fluorescence of the K series of iodine (28.5 keV) was measured with a scintillation counter having a 6-mm thick NaI/Tl crystal. The characteristic radiation of iodine was separated by means of a single-channel, pulse-amplitude analyzer covering the total width of the K-peak iodine. Measurement time was 1 min. A calibration curve of the whole range of iodine contents investigated was made from standard samples of iodic acid (HIO<sub>3</sub>).

In the absorption method the X-ray source was <sup>241</sup>Am and the energy was 60 keV. The detector was a scintillation counter with a 6-mm thick NaI/Tl crystal. Absorption measurements were made by means of a single-channel, pulse-amplitude analyzer in the energy channel of 5 keV covering 60 keV. Calibration curves were again prepared from suitable concentrations of iodic acid. The authors claim the fluorescence technique is the method of choice.

Analyses take less than 5 min, and the relative error is claimed to be 1-3%, depending on iodine content.

Mikolajek et al.<sup>44</sup> used the method of retrograde dispersion of beta particles to assay contrast media. <sup>208</sup>Tl, with about 3  $\mu$ Ci activity deposited on a ring was used as the source of radiation. A calibration curve showing the number of scattered electrons vs. concentration was established. Results by this method are in good agreement with determinations made by conventional methods.

### 6.8 Flame Photometry

Shamotienko<sup>47</sup> determined sodium iodipamide in pharmaceutical preparations by measuring the sodium content by flame photometry. Prior to analysis, the iodipamic acid was precipitated by acidifying the solution with 2N hydrochloric acid, and was then separated by filtration. Determinations for the filtrate were evaluated from a calibration curve of sodium chloride in the range of 4.5 - 8.5 mg %.

This procedure lacks specificity, since it is dependent on the content of an atom not associated with the activity of iodipamide. Many formulations are also pH adjusted with sodium hydroxide and contain other sodium compounds, e.g., sodium citrate, sodium edetate, as excipients. In these cases, this method would give erroneously high values.

### 7. Drug Metabolism

Iodipamide has been demonstrated to be excreted largely in the unchanged form<sup>12,16,17</sup>. Langecker et al.<sup>12</sup> recovered 70% of the unchanged compound in the bile of rabbits within 6 hr after dosing. Because of its low pKa (3.5)<sup>24</sup> and high molecular weight, iodipamide is not reabsorbed after its excretion in the bile<sup>18,19</sup>. Strickler et al.<sup>20</sup> reported evidence for the metabolic conversion of iodipamide to an unidentified product. Hydrolysis of the amide linkage was postulated as a possibility<sup>21</sup>.

Deiodination of iodipamide was studied in man<sup>21</sup> and was found to be less than 1% of the given dose. Pileggi

et al.<sup>22</sup> described a paper chromatographic screening test for determination of iodipamide and iodide in sera (Section 6.61). A method for the removal of iodipamide from blood sera by Craig countercurrent distribution was described by Strickler et al.<sup>20</sup> (Section 5.3).

McChesney<sup>23</sup> reviewed the literature through February 1968 in a chapter entitled, "The Biotransformation of Iodinated Radiocontrast Agents."

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# **METHADONE HYDROCHLORIDE**

*Rafik H. Bishara*

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## 1. Description

### 1.1 Nomenclature

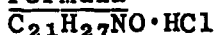
6-Dimethylamino-4,4-diphenyl-3-heptanone hydrochloride.

1,1-Diphenyl-1-(2-dimethylaminopropyl)-2-butanone hydrochloride.

4,4-Diphenyl-6-dimethylamino-3-heptanone hydrochloride.

6-Dimethylamino-4,4-diphenylheptan-3-one hydrochloride.

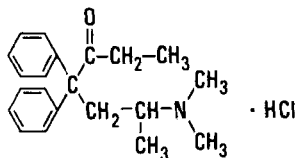
### 1.2 Formula



### 1.3 Molecular Weight

345.92

### 1.4 Structure



### 1.5 Appearance, Color, Odor, and Taste

White, essentially odorless powder.

Bitter taste followed by stinging sensation.

### 1.6 Proprietary Names

dl-Form: Adanon hydrochloride; Algidon; Algolysin; Amidon hydrochloride; AN-148; Butalgin; Depridol; Diadone; Diaminon hydrochloride; Dolophine hydrochloride; Eptadone; Fenadone; Heptadon hydrochloride; Hoechst 10,820; Ketalgin hydrochloride; Mecodin; Mephenon;

Miadone; Moheptan; Phenadone hydrochloride; Physeptone hydrochloride; Polamidon hydrochloride; Symoron; Zefalgin.

l-Form: Levadone; Levothy1.

## 2. Physical Properties

### 2.1 Melting Range

dl-Form:	mp	235.0°C <sup>1</sup>
	mp	232.5 - 233.0°C <sup>2</sup>
	mp	233.0 - 236.0°C <sup>3</sup>
	mp	236.0 - 236.5°C <sup>4</sup>
l-Form:	mp	241.0°C <sup>1</sup>
	mp	245.0 - 246.0°C <sup>4</sup>

### 2.2 Solubility<sup>1,3,4,5</sup>

Racemic methadone hydrochloride is very soluble in water (12 g/100 ml), soluble in alcohol (8 g/100 ml), in isopropanol (2.4 g/100 ml), and in chloroform; practically insoluble in ether and in glycerine.

The l-form of methadone hydrochloride has similar solubility to the racemic form in alcohol, in chloroform and in ether.

### 2.3 Optical Rotation

No optical rotation is observed with the racemic methadone hydrochloride. For the l-form the following optical rotations are reported:

$$[\alpha]_D^{20} -145^\circ \quad (C = 2.5)^1$$

and

$$[\alpha]_D^{20} -169^\circ \quad (C = 2.1 \text{ in alcohol})^{1,4}.$$

### 2.4 pH Range

The pH of a 1% solution is between 4.5 and 6.5.<sup>1,3,5</sup>

### 2.5 Dissociation Constant (pKa)

Levi et al.<sup>6</sup> reported the pKa of methadone hydrochloride, in water at 20°C., to

be 8.25. Other data on the dissociation constant of methadone are reported by Marshall<sup>7</sup> and Beckett.<sup>8</sup>

## 2.6 Partition Coefficient

Partition coefficients of dl-methadone in heptane/pH 7.4 buffer and chloroform/pH 7.4 buffer at 25°C. are 0.84 and 14.56 respectively.<sup>9</sup> Misra and Mule<sup>10</sup> reported 57.3 and 28.3 to be the partition coefficients of the l- and d-isomers, respectively, in octanol/pH 7.4 buffer. No experimental details are given to explain this difference between the two optical isomers.

## 2.7 Differential Thermal Analysis<sup>11</sup>

A differential thermal analysis, DTA, of methadone hydrochloride was performed using a DuPont 900 Differential Thermal Analyzer at a heating rate of 20°C. per min. and a nitrogen atmosphere. The thermogram (Figure 1) shows an endotherm at 235°C., which appears to be a melt, followed immediately by decomposition.

## 2.8 Thermogravimetric Analysis<sup>11</sup>

A thermal gravimetric analysis, TGA, of methadone hydrochloride was performed using a DuPont 950 Thermogravimetric Analyzer at a heating rate of 5°C. per min. and a nitrogen atmosphere. The thermogram (Figure 2) shows a weight loss beginning at 156°C. and continuing through decomposition.

## 2.9 Optical and Crystallographic Properties

The crystals of methadone hydrochloride prepared by Huback and Jones<sup>12</sup> for optical examination and identification were obtained by cooling a warm saturated aqueous solution of the compound. The single crystals were mounted on a stage goniometer to measure the principal refractive indices. A rotatory stage was used to measure all angles. Diamond-shaped crystals resting on an end face are reported. These crystals show symmetrical extinction, give an

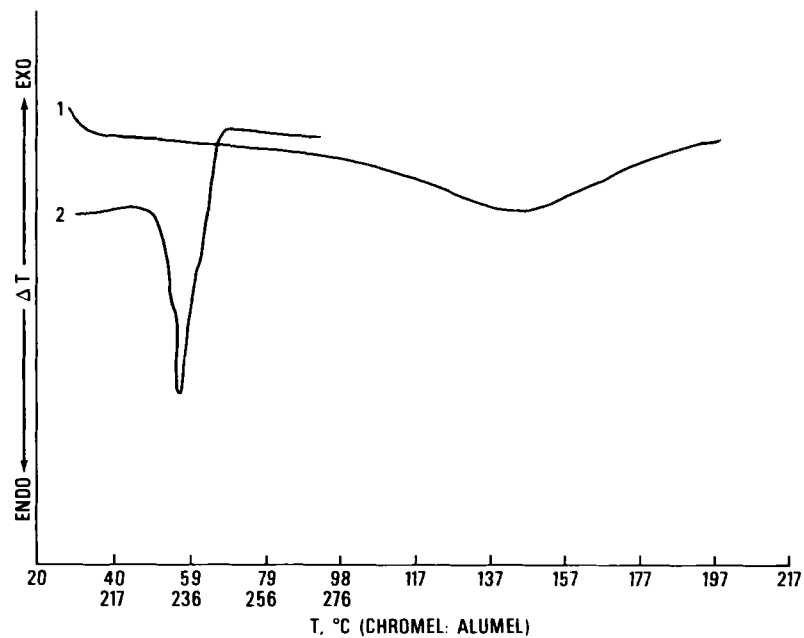


Figure 1. DTA-thermogram of methadone hydrochloride taken on a DuPont 900 Differential Thermal Analyzer



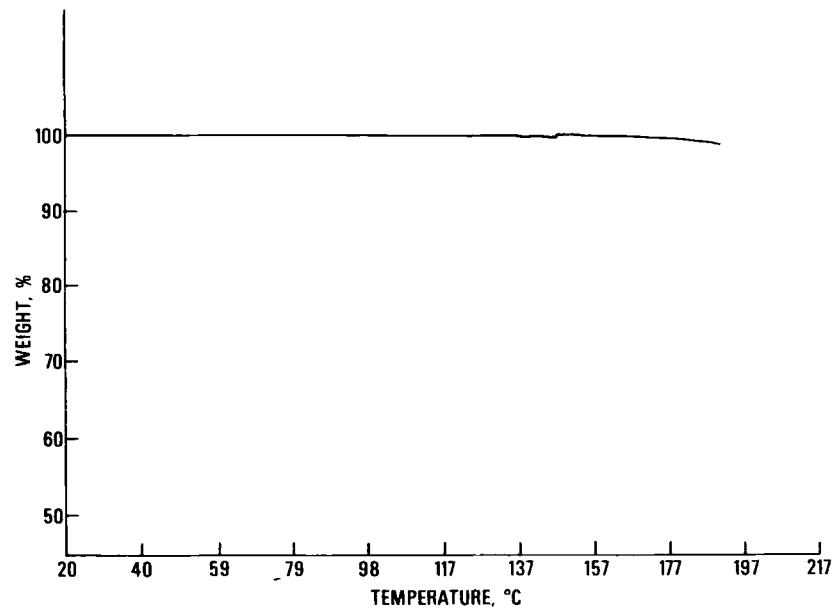


Figure 2. TGA-thermogram of methadone hydrochloride taken on a DuPont 950 Thermogravimetric Analyzer

interference figure that shows the obtuse bisectrix at one edge of the field, and their acute angle of  $62^\circ$  is reliable for characteristic diagnosis. Table 1 summarizes the optical and crystallographic data of racemic methadone hydrochloride.

The single crystal data for dl-methadone hydrochloride, tabulated by Barnes and Forsyth,<sup>13</sup> indicate that the space group of this compound is Cc or C2/c. The authors listed  $a = 16.26$ ,  $b = 9.76$ , and  $c = 25.74$  Å. The monoclinic angle was measured as  $74^\circ$  (i.e.,  $\beta = 106^\circ$ ). The space group extinction of C2/c is favored by the presence of eight molecules per cell ( $z = 8$  molecules/cell). A density of 1.178 g./ml. (average of 8 measurements) was observed for crystals from different preparations ( $P_{\text{calcd.}} = 1.171$  g./ml.).

## 2.10 X-Ray Powder Diffraction

The x-ray diffraction powder data of dl-methadone hydrochloride obtained by Barnes and Sheppard<sup>14</sup> using filtered  $\text{CoK}\alpha$  ( $\lambda = 1.790$  Å) radiation are in very good agreement with those obtained by Hubach and Jones,<sup>12</sup> for a sample from a different commercial source, with filtered  $\text{CuK}\alpha$  ( $\lambda = 1.542$  Å) radiation. With copper radiation, the spacings of the 3 strongest lines of the pattern are 7.46 Å (v.v.s.), 4.55 Å (v.v.s.), and 6.45 Å (v.s.) with cobalt radiation the 3 strongest spacings of the pattern are 4.57 Å (100), 7.50 Å (90), and 6.48 Å (70), thus merely interchanging the "first" and "second" lines.

Barnes and Sheppard<sup>14</sup> drew the attention to the fact that while the pattern of the dl-methadone hydrochloride is not the same as that of the d- and the l- isomers, the patterns of the free base in d-, l-, and dl- forms are identical. The x-ray powder diffraction data of dl-methadone hydrochloride reported by these authors<sup>14</sup> are shown below:

TABLE 1

OPTICAL AND CRYSTALLOGRAPHIC PROPERTIES OF  
RACEMIC METHADONE HYDROCHLORIDE\*

Crystal system	Monoclinic, Class 3, only a plane of symmetry; acute angle $\beta = 74^\circ$
Optic orientation	$\beta$ vibration direction is parallel to crystallographic axis b. Plane of symmetry contains axial plane. $\alpha$ direction is acute bisectrix which is nearly perpendicular to crystallographic axis c.
374 Refractive indices, 5893A.; 25°C.	$\alpha = 1.5713 \pm 0.0005$ , $\beta = 1.6232 \pm 0.0005$ , $\gamma = 1.6360 \pm 0.0005$ , $\alpha' = 1.5760 \pm 0.0005$ from crystals resting on an end face
Optic axial angle	
Observed	$2E = 90^\circ \pm 1^\circ$ by calibrated micrometer eyepiece
Calcd. from $\sin V =$ $\frac{\sin E}{1.623}$	$2V' = 52^\circ$
Observed	$2V = 52^\circ$ by rotating from one optic axis to the other on goniometer

(continued ...)

TABLE 1 (concluded)

Calcd. from indices

$$\cos^2 V = \frac{\gamma^2 (\beta^2 - \alpha^2)}{\beta^2 (\gamma^2 - \alpha^2)} \quad 2V = 52^\circ \text{C.}$$

Optical character

Negative

Dispersion

( $r < v$ ) distinct on both optic axes

---

\*Reproduced by permission.<sup>12</sup>

$d(\text{\AA})$	$I/I_1$	$d(\text{\AA})$	$I/I_1$
12.4	25	3.10	30
8.25	10	3.03	3
7.87	5	2.97	10
7.50	90	2.92	3
6.48	70	2.84	5
6.20	1	2.75	20
5.92	20	2.68	15
5.70	5	2.60	3 B
4.72	30	2.53	20
4.57	100	2.48	2
4.34	40	2.30	5
4.20	3	2.23	1
4.14	20	2.16	2
4.00	20	2.11	8
3.87	20	2.08	3
3.71	5 BB	2.04	15
3.49	20 B	1.99	2
3.33	5	1.92	3
3.20	25	1.67	2
3.14	1 B		

## 2.11 Ultraviolet Spectrum

A scan of **methadone hydrochloride** in ethanol at a concentration of 0.27 mg./ml., ( $7.8 \times 10^{-4}$  M) on a Cary 15 spectrophotometer, from 400 to 210 nm. (Figure 3) shows maxima at 254, 259, 265, and 293 nm. The corresponding molar absorptivities ( $\epsilon$ ) of these maxima are 410, 485, 460, and 470 respectively.

Hubach and Jones<sup>12</sup> reported that an alcoholic solution of methadone hydrochloride exhibited two characteristic electronic absorption bands at  $\lambda_{\max} = 294$  nm. ( $\epsilon = 460$ ) and the aromatic band at  $\lambda_{\max} = 259$  nm. ( $\epsilon = 480$ ). In water solution, the long wavelength maximum is shifted to 292 nm. and the molar absorptivity is increased to  $\epsilon = 520$ . The spectrum of the free base, methadone, using ethanol or hexane as a solvent, is essentially the same in the region of the 294 nm. band.

# METHADONE HYDROCHLORIDE

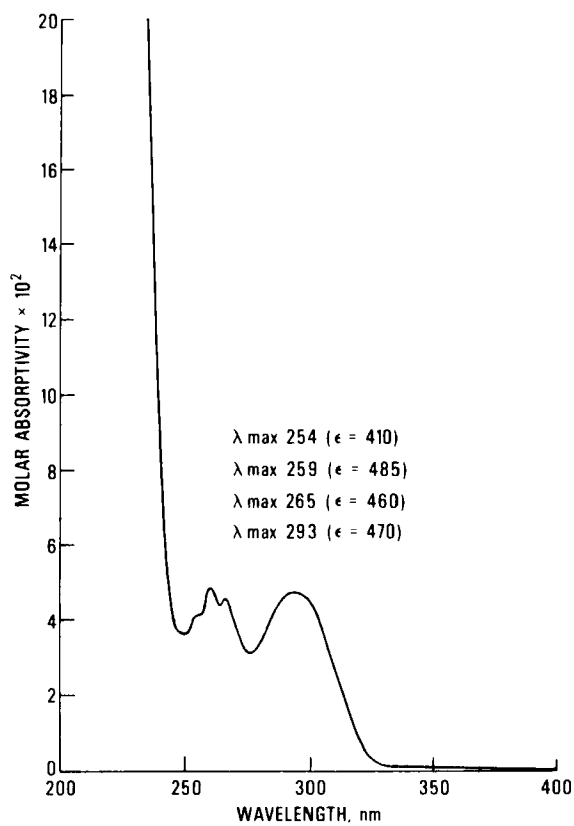


Figure 3. Ultraviolet spectrum of methadone hydrochloride in ethanol taken on a Cary 15 Spectrophotometer

However, the molar absorptivities are  $\epsilon = 760$  in alcohol and  $\epsilon = 830$  in hexane.

The ultraviolet absorption spectra data of Mule<sup>15</sup> on methadone in 0.1 N hydrochloric acid showed  $\lambda_{\max}$  at 292 nm. ( $\epsilon = 554$ ),  $\lambda_{\min}$  at 275 nm. ( $\epsilon = 372$ ). Data obtained in ethylene dichloride containing 25% isobutanol (v/v) are  $\lambda_{\max}$  at 295 nm. ( $\epsilon = 433$ ) and  $\lambda_{\min}$  at 280 nm. ( $\epsilon = 390$ ).

Ultraviolet absorption spectra at reduced temperatures of methadone nitrile and isomethadone nitrile are presented by Sinsheimer *et al.*<sup>16</sup>

## 2.12 Infrared Spectrum

A scan of methadone hydrochloride in a potassium bromide pellet on a Beckman IR-12 spectrophotometer is shown in Figure 4. Underbrink<sup>17</sup> assigned the following bands to the methadone hydrochloride IR spectrum:

- |  |  |
|--|--|
| a. 710-770 $\text{cm}^{-1}$                    | characteristic for aromatic carbon-hydrogen out of plane bending.                              |
| b. 900-1200 $\text{cm}^{-1}$                   | fingerprint region; due to skeletal frequencies and aromatic carbon-hydrogen in plane bending. |
| c. 1300-1500 $\text{cm}^{-1}$                  | characteristic for methylene and methyl bending.   |
| d. 1450, 1490, 1580, and 1600 $\text{cm}^{-1}$ | characteristic for aromatic ring frequencies.  |
| e. 1708 $\text{cm}^{-1}$                       | characteristic for carbonyl stretching.  |

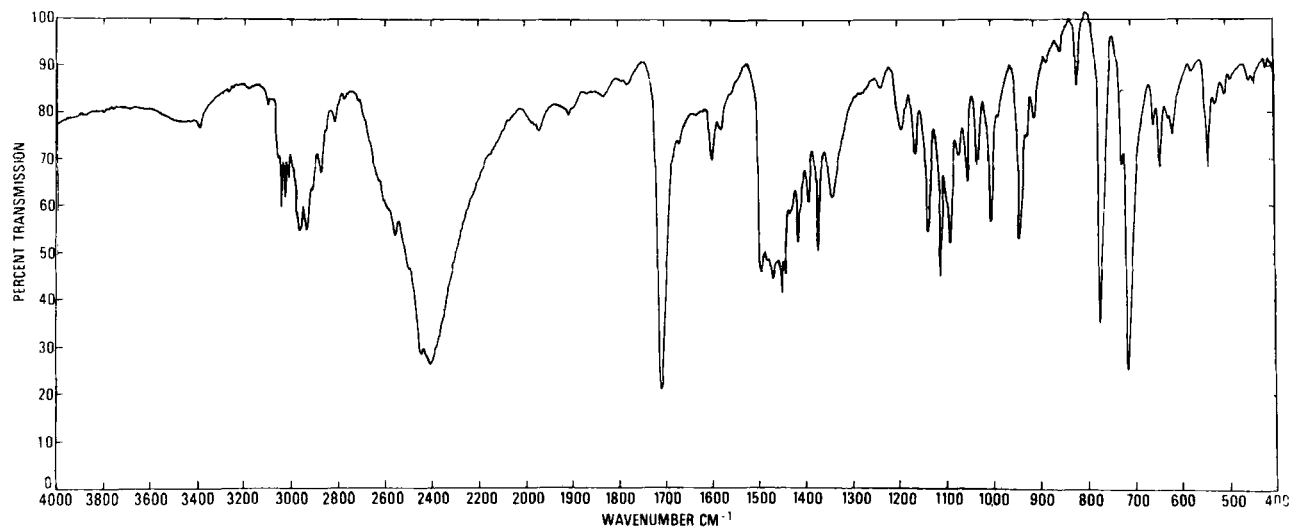


Figure 4. Infrared spectrum of methadone hydrochloride taken in a KBr pellet on a Beckman IR-12 Spectrophotometer



- |                                |   |
|--------------------------------|---|
| f. 2400 cm. <sup>-1</sup>      | characteristic for<br>tertiary amine<br>hydrochloride           |
| g. 2810-3000 cm. <sup>-1</sup> | characteristic for<br>aliphatic carbon-<br>hydrogen stretching. |
| h. 3000-3080 cm. <sup>-1</sup> | characteristic for<br>aromatic carbon-<br>hydrogen stretching.  |

### 2.13 Nuclear Magnetic Resonance Spectrum

The NMR spectrum of methadone hydrochloride in CDCl<sub>3</sub> containing tetramethylsilane as internal standard on a Varian Associates HA-100 is shown in Figure 5. The spectral assignments<sup>18</sup> are summarized in Table 2. The chemical shifts are measured in p.p.m. downfield from tetramethylsilane. The multiplicity of the peaks, and the approximate coupling constants (J) are given in Hz where appropriate.

The methylene and methine protons of Group 3 and Group 4, respectively, were identified by decoupling. The two methylene protons of Group 6 are nonequivalent probably because of conformational effects.<sup>19,20</sup> Their chemical shifts are assigned at approximately 2.30 and 3.15 p.p.m. by the process of elimination and integration. The protons of the N-methyl groups are nonequivalent due to the relative proximity of each methyl group to the deshielding cone of the phenyl group and appear as a pair of doublets ( $J \simeq 4 \text{ Hz}$ ) centered at 2.75 p.p.m.

Discussion of the NMR and PMR of methadone and some related substances, and the application of this technique for stereochemical and optical isomerism problems are found in the literature.<sup>19-23</sup>

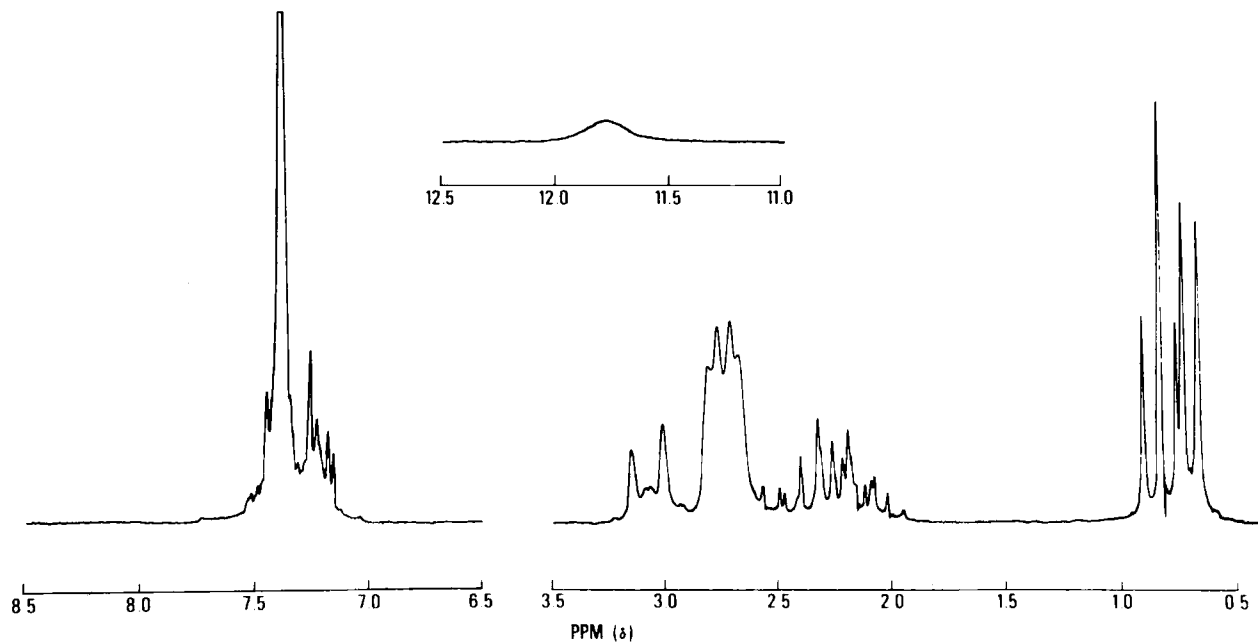


Figure 5. Nuclear magnetic resonance spectrum of methadone hydrochloride in  $\text{CDCl}_3$  taken on a Varian Associates HA-100 Spectrometer

TABLE 2

## NMR SPECTRAL ASSIGNMENTS OF METHADONE HYDROCHLORIDE

	<u>Group</u>	<u>Multiplicity</u>	<u>Chemical Shift (p.p.m.)</u>	<u>J(Hz)</u>
1.	$\text{CH}_3\text{-CH-}$   $\text{N(CH}_3)_2$	Doublet	0.70	6.5
2.	$\text{CH}_3\text{-CH}_2\text{-C-}$    O	Triplet	0.83	7
3.	$\text{CH}_3\text{-CH}_2\text{-C-}$    O   $\text{N(CH}_3)_2$		Approx. 2.30	
4.	$\text{CH}_3\text{-CH-CH}_2\text{-}$   $\text{N(CH}_3)_2$	Multiplet	Approx. 3.04	
5.	$\text{CH}_3\text{-CH-}$   $\text{N(CH}_3)_2$	Multiplet	2.75	

(continued ...)

TABLE 2 (concluded)

	<u>Group</u>	<u>Multiplicity</u>	<u>Chemical Shift (p.p.m.)</u>	<u>J(H<sub>Z</sub>)</u>
6.	$  \begin{array}{c}  \text{C}_6\text{H}_5 \\    \\  -\text{C}-\underline{\text{CH}_2}-\text{CH}- \\    \\  \text{C}_6\text{H}_5  \end{array}  $		One proton Approx. 2.30 One proton Approx. 3.15	
7.	$  \begin{array}{c}  -\text{C}-\text{CH}_2- \\    \\  (\text{C}_6\text{H}_5)_2  \end{array}  $	Multiplet	7.40	
8.	$  \begin{array}{c}  + \\  \text{H}-\text{N}  \end{array}  $	Broad singlet	11.8	

## 2.14 Mass Spectrum

The relative mass fragmentation pattern of methadone was obtained<sup>24</sup> using an LKB-9000 combined gas chromatograph-mass spectrometer (GCMS). A four-foot siliconized glass column (2.5 mm. I.D.) packed with 1% W-98 methylvinyl silicon gum rubber on 80-100 mesh Gas Chrom Q was employed as the GC column. The column temperature was 170°C. and the carrier gas (helium) flow was 40 ml./min. An electron energy of 70 eV was used for ionization. The computerized mass fragmentation pattern shown in Figure 6 was obtained by using a Hewlett Packard 2100 computer interfaced directly to the GCMS. The assignments and compositions are as follow:

<u>m/e</u>	<u>Assignment</u>	<u>Composition</u>
309	M <sup>+</sup>	C <sub>21</sub> H <sub>27</sub> NO
294	(M-CH <sub>3</sub> ) <sup>+</sup>	C <sub>20</sub> H <sub>24</sub> NO
265	(M-N(CH <sub>3</sub> ) <sub>2</sub> ) <sup>+</sup>	C <sub>19</sub> H <sub>21</sub> O
223	(M-CH <sub>2</sub> -CH-CH <sub>3</sub> ) <sup>+</sup>   N(CH <sub>3</sub> ) <sub>2</sub>	C <sub>16</sub> H <sub>15</sub> O
165		C <sub>13</sub> H <sub>9</sub>
72	(-CH-CH <sub>3</sub> ) <sup>+</sup>   N(CH <sub>3</sub> ) <sub>2</sub>	C <sub>4</sub> H <sub>10</sub> N
57	(-CO-CH <sub>2</sub> -CH <sub>3</sub> ) <sup>+</sup>	C <sub>3</sub> H <sub>5</sub> O
44	(-N(CH <sub>3</sub> ) <sub>2</sub> ) <sup>+</sup>	C <sub>2</sub> H <sub>6</sub> N
29	(-CH <sub>2</sub> -CH <sub>3</sub> ) <sup>+</sup>	C <sub>2</sub> H <sub>5</sub>

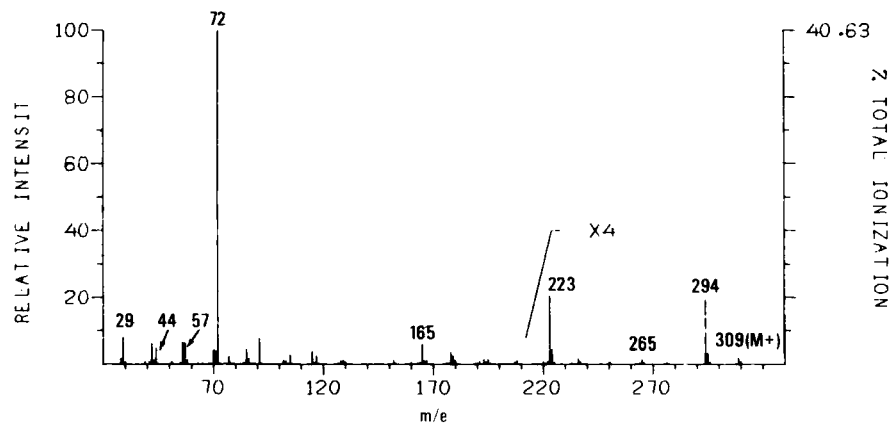


Figure 6. Relative mass fragmentation pattern of methadone obtained by using an LKB-9000 combined gas chromatograph-mass spectrometer (GCMS). Data courtesy of Sullivan, H. R.<sup>24</sup>

The above data are in good agreement with the high resolution mass spectrum<sup>25</sup> of methadone hydrochloride obtained by using a CEC21-110A mass spectrometer with photoplate recording. The assignments of the prominent ions are given in Table 3.

The mass spectrum of methadone, the most abundant peaks and the metastable ions are reported by Fales et al.<sup>26</sup> The identification of methadone by isobutane chemical ionization mass spectrometry is presented by Milne et al.<sup>27</sup>

### 3. Synthesis, Structure and Resolution

#### 3.1 Synthesis and Confirmation of Structure

The report of the United States Department of Commerce<sup>28</sup> about Amidone (methadone), the new German analgesic drug no. 10820, includes the method given by the German chemists for its synthesis. In this method, 1-chloro-2-propanol is added to an aqueous solution of diethylamine and sodium hydroxide to prepare 1-dimethylamino-2-propanol [1]. To [1] a solution of thionyl chloride in benzene is added to form 1-dimethylamino-2-chloropropane [2]. Compound [2] is then dropped on a cool mixture of sodamide and diphenylacetoneitrile and the temperature is allowed to rise. The solution is refluxed for 15 minutes, cooled, poured on water, and the water is removed. The benzene solution is acidified with hydrochloric acid, the aqueous acidic layer is made alkaline with sodium hydroxide, and the product, 1-dimethylaminopropyl-2-diphenylacetoneitrile [3], is dissolved in xylene and added to a solution of ethylmagnesium bromide, heated, and poured over acidified water to separate the hydrobromide of the ketone. The latter compound is dissolved in warm water and made alkaline to yield an oily base, methadone, which is crystallized from methanol. The hydrochloride of methadone is prepared by dissolving the base in alcohol, alcoholic hydrogen chloride is added, and upon cooling, the material crystallizes.

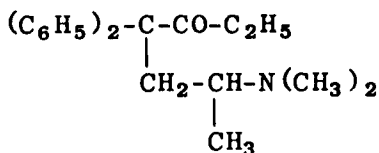
TABLE 3

## HIGH RESOLUTION MASS SPECTRUM ASSIGNMENTS OF METHADONE HYDROCHLORIDE

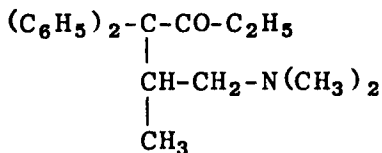
<u>Calculated Mass</u>	<u>Theoretical Mass</u>	<u>Emperical Formula</u>			
		<u>C</u>	<u>H</u>	<u>N</u>	<u>O</u>
309.2061	309.2093	21	27	1	1
294.1854	294.1858	20	24	1	1
265.1586	265.1592	19	21	0	1
236.1433	236.1439	17	18	1	0
223.1121	223.1123	16	15	0	1
179.0846	179.0861	14	11	0	0
178.0779	178.0782	14	10	0	0
165.0699	165.0704	13	9	0	0
117.0711	117.0704	9	9	0	0
115.0543	115.0548	9	7	0	0
91.0552	91.0548	7	7	0	0
85.0896	85.0892	5	11	1	0
72.0814	72.0813	4	10	1	0
71.0745	71.0735	4	9	1	0
70.0653	70.0657	4	8	1	0



It was noted<sup>28,29</sup> that the above-mentioned synthesis would not be expected to lead to methadone [4], but to the formation of the isomeric structure of isomethadone [5]:



[4]



[5]

Schultz *et al.*<sup>29</sup> established and proved structure [4] for methadone. In their synthesis, when diphenylacetoneitrile is reacted with 1-dimethylamino-2-chloropropane [2] either in the presence of sodamide<sup>28</sup> or potassium t-butoxide, the product is a mixture of approximately equal amounts of the isomeric nitriles [3] and [6]. The 2,2-diphenyl-4-dimethylaminopentanenitrile [6] reacts smoothly with ethylmagnesium bromide to give methadone [4]. Treatment of 2,2-diphenyl-3-methyl-4-dimethylaminobutanenitrile [3] with the Grignard's reagent does not give the methadone isomer [5] but a dibasic product [7] which appears to be the corresponding ketimine. Although the ketimine structure is supported by analytical data, the ordinary condition of hydrolysis fails to give the ketone.<sup>30</sup> The structure of the isomeric nitriles, and hence the structure of methadone, were established by decomposition of the quaternary bases derived from the methiodides of the nitriles by treatment with silver oxide. A summary of this synthesis is illustrated in Figure 7.

Brode and Hill<sup>31</sup> reported the rearrangement of the isomeric 1,2-dimethylaminochloropropanes, derived from the chlorination of 1-dimethylamino-2-propanol [8] and 2-dimethylamino-1-propanol [9], through the

METHADONE HYDROCHLORIDE

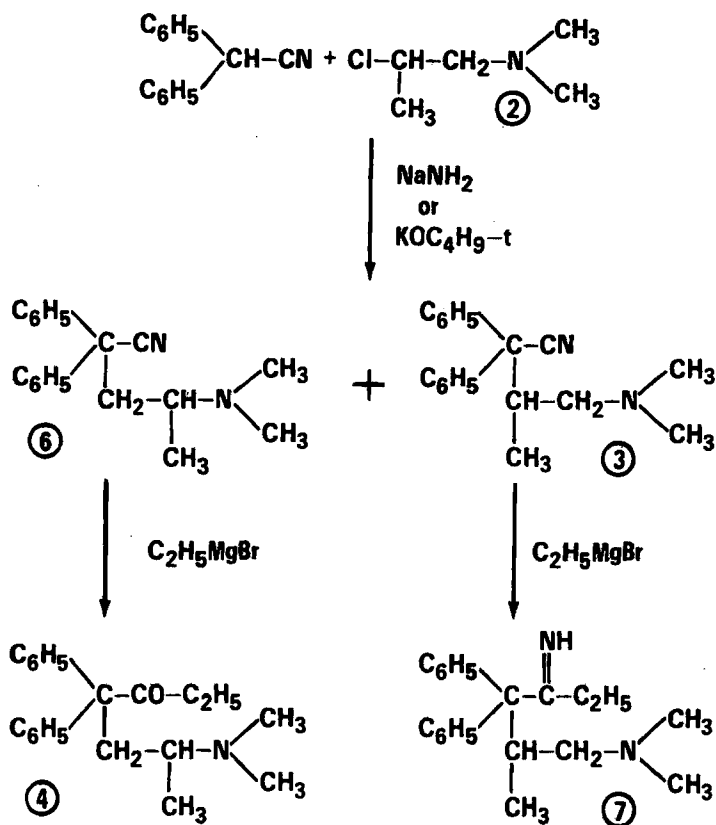


Figure 7. Synthesis of methadone according to Schultz et al.<sup>29</sup>

ethylene immonium ion.<sup>32</sup> Their data also show the conversion of [8] to [9] in low yields.

To avoid the technical difficulties due to the formation of the isomeric aminonitriles, a new synthesis was developed by Easton et al.<sup>33</sup> in which diphenylacetonitrile is condensed with propylene oxide in the presence of sodium amide to yield 3,3-diphenyl-5-methyltetrahydro-2-furanoneimine [10]. When [10] is treated with phosphorus tribromide, the product is 4-bromo-2,2-diphenylpentanenitrile [11]. On condensing [11] with dimethylamine, Compound [6] is formed. Methadone [4] is prepared from [6] by the action of ethylmagnesium bromide as discussed previously.<sup>28</sup> The yields of the aminonitrile from the halonitrile is below 10%. The major product always formed is an unsaturated nitrile, presumably 2,2-diphenyl-3-pentenenitrile [12] or a mixture of [12] and 2,2-diphenyl-4-pentenenitrile [13].

The structure of methadone established by Schultz et al.<sup>29</sup> was confirmed by the following series of reactions. Compound [6] is degraded by exhaustive methylation (methyl iodide, silver oxide, and heating) to an unsaturated nitrile [12], [13] or a mixture which is hydrolyzed without purification to yield the lactone of 2,2-diphenyl-4-hydroxypentanoic acid [14]. The hydrolysis of [13] forms the same lactone [14]. Long standing of the hydrochloride of Compound [10] in aqueous solution gives the lactone [14]. These facts are accounted for by the straight structure of the aminonitrile [6]. Figure 8 shows the synthesis and confirmation of structure according to Easton et al.<sup>33</sup>

The preparation of some isomers,<sup>34</sup> analogs,<sup>35-39</sup> and related substances<sup>40-44</sup> to methadone is reported in the literature. Tolbert et al.<sup>45</sup> synthesized dl-methadone labeled with <sup>14</sup>C- in either the 1 or 2 position.

METHADONE HYDROCHLORIDE

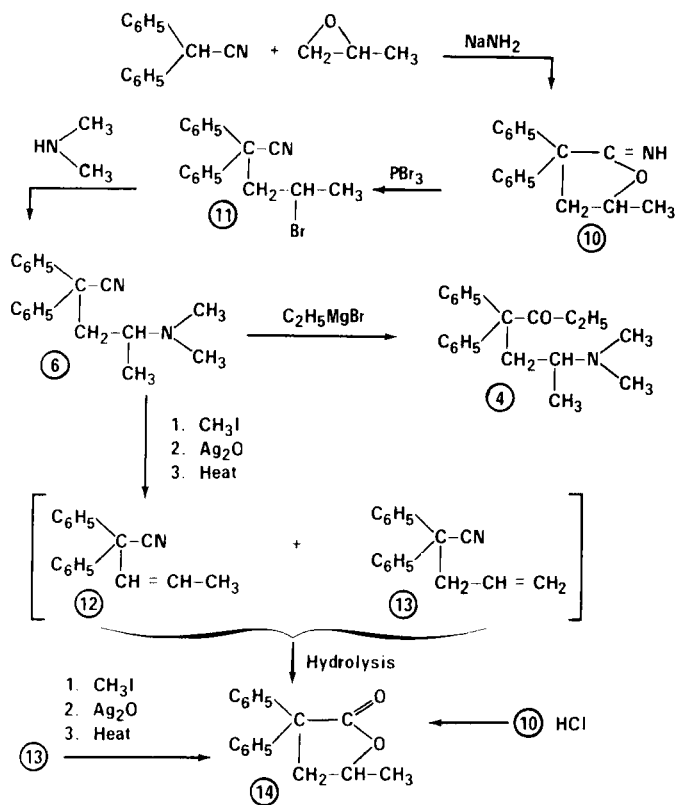


Figure 8. Synthesis and confirmation of methadone structure according to Easton et al.<sup>33</sup>

### 3.2 Resolution

Methadone has one assymetric carbon atom and therefore can exist as dextro or levo forms or as racemic mixture. The optical resolution of methadone is reported by Brode and Hill,<sup>46</sup> Larsen et al.,<sup>47</sup> and Thorp et al.<sup>48</sup> through the use of d-tartaric acid. Howe and Sletzing,<sup>49</sup> and Howe and Tishler<sup>50</sup> resolved dl-methadone, or its hydrochloride, by forming the easily purified, water-insoluble d- $\alpha$ -bromocamphor- $\pi$ -sulfonate of the d-isomer. Pure d-methadone is precipitated by slow addition of water. The l-form is obtained, from the mother liquor, by forming the d-tartrate salt. When the l-methadone is desired, the d-isomer is removed from a solution in butyl alcohol as the p-nitrobenzoyl-L-glutamate. The use of  $\alpha$ -bromocamphor- $\pi$ -sulfonic acid and p-nitrobenzoyl-L-glutamic acid as the resolving agent reduces the excessive crystallization time and substantially increases the yields. Zaugg<sup>51</sup> patented a special apparatus to provide a new physical method for simultaneous resolution of both optical isomers of dl-methadone. Zaugg explains that "this invention is based on the knowledge that a seed crystal of the dextro-rotatory isomer will attract the d-isomer in saturated solution, and when the degree of saturation of the solute in the solution is increased, the d-isomer will tend to crystallize out on the d-isomer seed crystal. At the same time, a portion of the l-isomer will tend to crystallize out on the l-isomer seed crystal. This process will continue so long as the solution is supersaturated with the composition or solute and seeded crystals will grow to substantial size. At the conclusion of the operation, it will be found that relatively pure crystals of the d-isomer and l-isomer will have been grown on the seed crystals."

### 4. Reactivity and Stability

The relatively low reactivity of the carbonyl group of methadone [1] is indicated by not giving the semicarbazone under the usual conditions and

resisting reduction with aluminum isopropoxide or sodium amalgam.<sup>36</sup> The corresponding carbinol [2] is formed with platinum oxide. Acetylation of [2] yields the O-acetyl derivative [3-a]. Reaction of [2] with chlorinating agents (thionylchloride or phosphorus pentachloride) leads to the formation of a mixture of 6-dimethylamino-4,4-diphenyl-2-heptene [4] and 3-chloro-6-dimethylamino-4,4-diphenylheptane [5]. Alkaline cleavage of the ethyl keto group results in the formation of 3-dimethylamino-1,1-diphenylbutane [6]. Compound [6] is also formed by refluxing 4-dimethylamino-2,2-diphenylpentanenitrile [7] with potassium hydroxide and triethyleneglycol. Hydrogenation of the resulting olefin [9] from the Hofmann degradation of [8], the methiodide of [6] (which is also formed by alkali treatment of the methiodide of [1]), gives 1,1-diphenylbutane [10]. The latter compound is also prepared from ethyl butyrate [11] via 1,1-diphenyl-1-butanol [12] which is hydrogenated to [10] with palladium-charcoal or palladium-barium sulfate catalyst in the presence of acetic acid containing traces of perchloric acid. Alkali treatment of  $\alpha,\alpha$ -diphenylvaleronitrile [13] gives a low yield of [10] along with  $\alpha,\alpha$ -diphenylvaleric acid [14]. Figure 9 shows these reactions. In a later report by May and Perrine<sup>52</sup> the structures of [4] and [5] were proven to be 6-dimethylamino-3,4-diphenyl-3-heptene [15], and 4-chloro-6-dimethylamino-3,4-diphenylheptane [16], respectively. This is due to the Wagner's rearrangement of [2] to give 6-dimethylamino-3,4-diphenyl-4-heptanol [17], [15] and [16] depending upon the reaction conditions.

Irradiation with gamma rays (cobalt-60), ultraviolet, or thermal neutrons hardly affects the melting point of methadone hydrochloride.<sup>53,54</sup> However, the irradiation produces a brown color, changes of pH in solution, decreases specific rotation, and modifies the infrared spectrum. Additional spots appear

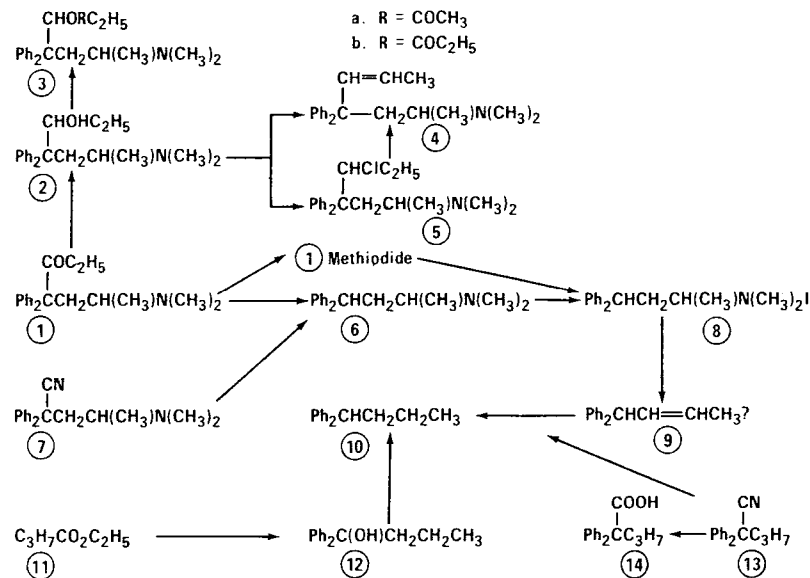


Figure 9. Some reactions of methadone.<sup>36</sup> Reproduced by permission.

on the thin layer chromatogram of the irradiated sample. Irradiation is less destructive to the solid material than to the aqueous solution.

Photolysis and radiolysis of methadone hydrochloride solution result in the formation of 3,3-diphenyl-2-ethylidene-5-methyltetrahydrofuran and 3-dimethylamino-1,1-diphenylbutene, respectively.<sup>55, 56</sup>

Storage of an organic solution of methadone free base at 30°C. shows the formation of methadone-N-oxide by thin layer chromatography and combined gas chromatography-mass spectrometry analyses.<sup>57</sup> The relative concentration of the chemical oxidation product, methadone-N-oxide, increases with time of storage.

## 5. Drug Metabolic Products and Pharmacokinetics

### 5.1 Absorption

Absorption of methadone is relatively prompt. Experiments with <sup>14</sup>C-methadone show appreciable concentrations of <sup>14</sup>C in plasma<sup>58</sup> and bile<sup>59</sup> within 10 minutes after subcutaneous injection of the labeled drug. Following subcutaneous injection of methadone in rats, 47% of the dose remains at the injection site after 1 hour,<sup>60</sup> 10-15% after 2-3 hours, 3% after 5 hours, and virtually none is present after 24 hours.<sup>61</sup> Seventy percent of a methadone dose administered by stomach tube to fasted rats disappears within 2 hours from the gastrointestinal tract.<sup>62</sup>

### 5.2 Distribution

Methadone mainly localizes in the liver, kidneys, lungs, and spleen. Blood, heart, brain, and muscle show only low levels.<sup>60-64</sup> Methadone is also concentrated in the adrenals and thyroid.<sup>58, 61, 65</sup> Using sensitive tracer techniques,<sup>65</sup> methadone levels of 0.6 to 0.9 µg./g. of various segments of the central nervous system are found 30 minutes after



subcutaneous administration of 3 mg./kg. This correlates well with the intensity and duration of the analgesic effect as demonstrated by the reaction time of the rat tail to thermal stimulus. Elliott et al.<sup>61</sup> reported high concentration of methadone in the gastrointestinal tract after subcutaneous administration of the drug. Considerable amounts of radioactivity are found in the placentae and fetuses of the pregnant rat after the administration of  $^{14}\text{C}$  labeled methadone.<sup>61</sup> The methadone concentration in the brain of the fetus is 2-3 times the concentration found in the maternal brain.<sup>66</sup>

Methadone appears to be firmly bound to tissue protein.<sup>67</sup> However, accumulation of the drug does not occur to any great extent. A large part of the methadone present in the whole animal is found in the carcass, mainly the skeleton, muscle, and bone.<sup>60,61</sup>

A distribution study of methadone in man<sup>68</sup> shows that the blood concentration of the drug is less than bile and urine concentrations. The kidney and liver concentrations are approximately equivalent. Brain tissue is the poorest source of methadone and lung the richest. Binding of methadone to human plasma albumin is reported by Olsen.<sup>69</sup>

### 5.3 Metabolism

The indication that the first two carbon atoms of methadone are not removed by oxidation was demonstrated by Elliott et al.<sup>61</sup> No  $^{14}\text{CO}_2$  is eliminated by rats given methadone labeled with  $^{14}\text{C}$  in position 2. Contrary to these early data, the recent work by Sullivan<sup>24</sup> shows that about 1% of the dose of  $^{14}\text{C}$  labeled methadone in position 2 is eliminated as  $^{14}\text{CO}_2$ . The presence of 4-dimethylamino-2,2-diphenylvaleric acid in urine of humans is also an indication of the side chain oxidation.<sup>57</sup> The liver appears to be the organ chiefly responsible for the metabolism of methadone.<sup>63,70-73</sup>

The major metabolites of methadone in humans are shown in Figure 10.<sup>57,74</sup> The primary metabolite of methadone [1] is formed by N-demethylation to yield the unstable N-desmethylnmethadone [2] which is cyclized<sup>75</sup> to 1,5-dimethyl-3,3-diphenyl-2-ethylidene pyrrolidine [3]. Further N-demethylation of [3] forms 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline [4]. Both [3] and [4] and their corresponding ring hydroxylated analogs, 2-ethylidene-1,5-dimethyl-3-(p-hydroxyphenyl)-3-phenylpyrrolidine [5] and 2-ethyl-5-methyl-3-(p-hydroxyphenyl)-3-phenyl-1-pyrroline [6], are detected in human urine.<sup>76-79</sup>

In a minor pathway, the keto group of methadone is enzymatically reduced<sup>59</sup> to form methadol [7] which is N-demethylated to yield normethadol [8] which is excreted in the urine.<sup>80</sup> In a relatively minor pathway the side chain of methadone is oxidized to form 4-dimethylamino-2,2-diphenylvaleric acid [9] which subsequently N-demethylates, in part, to a non-isolated intermediate, 4-methylamino-2,2-diphenylvaleric acid [10]. Ring closure (cyclization) of the intermediate [10] yields 1,5-dimethyl-3,3-diphenyl-2-pyrrolidone [11].

In addition to the previously mentioned phenolic metabolites [5] and [6], ring hydroxylated methadone [12] is also found in the urine of subjects maintained on methadone.<sup>57</sup> Methadone N-oxide [13] is found in urine from subjects receiving a single dose of methadone, in urine from addicts being treated with the drug,<sup>74</sup> and in urine of rats.<sup>10</sup> However, the work of Sullivan and Due<sup>57</sup> in humans implies that there is some question as to whether the methadone N-oxide is a true metabolite or an artifact caused by oxidation.

#### 5.4 Excretion

Way and Adler<sup>81</sup> indicated that less than 10% of methadone is excreted unchanged in the urine and in the feces. Urinary excretion studies show various concentrations of methadone

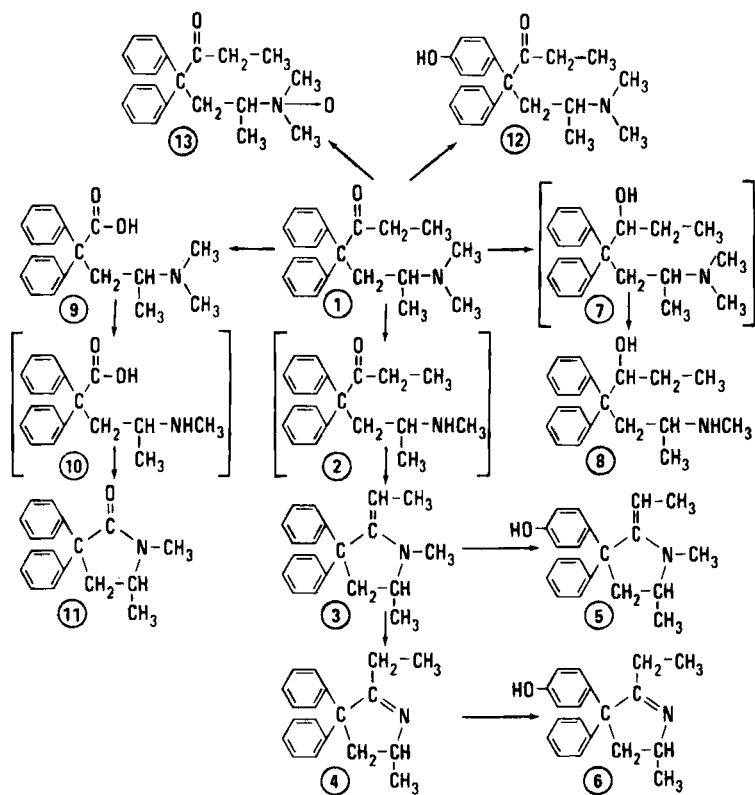


Figure 10. Major metabolites of methadone in humans.<sup>57,74</sup>

in urine. Recovery of 4% to 35% of the administered dose is reported.<sup>82-85</sup> Twenty-four hours after administration of methadone to rats, the unchanged drug found in urine and feces is 4-11% and 19-24% respectively from the administered dose.<sup>62</sup> However, Way *et al.*,<sup>82</sup> utilizing counter-current techniques, showed that the previous values are high and recommended a factor of 0.8 and of 0.25 for the correction of the urinary and fecal excretion respectively.

Biliary excretion is reported to be an important avenue for the elimination of methadone and its biotransformation products.<sup>59,63-65,82</sup>

The results of Baselt and Casarett<sup>9</sup> demonstrated that, in man, renal elimination may become the major excretory pathway after daily doses greater than 55 mg. Sixty percent of a 160 mg. dose of methadone per day is excreted as unchanged drug in urine. These results are in conflict with those of Sullivan and Due<sup>57</sup> who reported that a relatively small portion of an 80 mg. dose of methadone was found unchanged in the urine of heroin maintenance subjects. Urinary methadone excretion is markedly enhanced by acidification of the urine.<sup>9</sup> A sex difference in the pattern of excretion of methadone and its metabolites is found and is related to the rate of biotransformation of the drug.<sup>9</sup>

Methadone, metabolites [3] and [4] (See Figure 10), are present in sufficiently high concentration in human sweat to suggest that sweat may be a significant route of elimination of this drug.<sup>86</sup>

The mean apparent half-life of orally-administered methadone is 15 hours.<sup>87</sup> Following intramuscular administration, the half-life is 7.3 hours.<sup>88</sup> Subjects of a methadone maintenance program who receive a large oral dose of 100 or 120 mg. show a mean apparent half-life of methadone to be 25 hours.<sup>89</sup>

Analytical procedures for isolation, determination, and identification of methadone and its metabolites from biological fluids and tissues include colorimetric and photometric techniques following interaction with indicator dyes,<sup>60,62,64,67,70,82-84</sup> paper chromatography,<sup>72,90,91</sup> column chromatography,<sup>79,80</sup> gas chromatography,<sup>9,68,74,76-78,80,92</sup> thin layer chromatography,<sup>39,49,54,56,60,70</sup> repeated counter-current transfer,<sup>81</sup> radiotracer methods,<sup>45,58-61,65,72,80</sup> infrared,<sup>76,81,92</sup> nuclear magnetic resonance,<sup>74,76,78,92</sup> and combined gas chromatography-mass spectrometry.<sup>57,74,77-80</sup>

## 6. Identification

Methadone hydrochloride can be identified by virtue of its characteristic x-ray powder diffraction pattern, UV, IR, and NMR spectra (See 2.10, 2.11, 2.12, and 2.13). The characteristic melting point of about 160°C., or about 180°C. of the crystals formed by picrolonic acid and methadone is also useful as an identity test.<sup>3,5</sup> Addition of 2 ml. of methyl orange test solution<sup>5</sup> to a 0.5% methadone hydrochloride solution forms a yellow precipitate. The melting point of the water-washed residue, formed by adding excess sodium hydroxide solution<sup>3</sup> to a 5% solution of methadone, is about 76°C. Methadone hydrochloride reacts positively to the chloride characteristic test with silver nitrate.<sup>3,5</sup>

## 7. Microchemical Reactions

The microchemical identification of methadone through the formation of crystals with certain reagents has been reported.<sup>12,93-97</sup> Hubach and Jones<sup>12</sup> listed seven reagents which produced characteristic microscopic crystals from dilute aqueous methadone hydrochloride solutions. Equal drops of the reagent and the methadone solution are mixed on a microscopic slide and allowed to stand until crystals develop or all liquid evaporates. The crystals are then

observed and examined microscopically. The reagents used and the lowest concentration of methadone hydrochloride from which crystals are obtained are presented in Table 4 along with the description of the formed crystals.

Five modified cobalt (II) thiocyanate solutions give turquoise color with methadone.<sup>98</sup> A solution consisting of 0.8% w/v cobalt (II) thiocyanate in a (2:3) v/v mixture of methanol and 1% orthophosphoric acid (sp. gr. 1.75) gives a color response within 5 seconds from the addition of the reagent.

## 8. Methods of Analysis

### 8.1 Elemental Analysis (As $C_{21}H_{27}NO \cdot HCl$ )

<u>Element</u>	<u>% Theory<sup>1</sup></u>	<u>% Determined<sup>47, 49</sup></u>		
		<u>dl</u>	<u>d</u>	<u>l</u>
C	72.91	73.14	72.77, 72.90	73.06, 72.95
H	8.16	8.03	7.98, 8.36	8.23, 7.99
N	4.05	3.96	3.88	4.07
O	4.63			
Cl	10.25			

## 8.2 Titration

### 8.2.1 Non-Aqueous Titration

The tertiary amine group of methadone hydrochloride is directly titrated in non-aqueous medium, glacial acetic acid, in the presence of mercuric acetate to tie up the chloride ion. The technique of the non-aqueous titration of the drug using visual indicators,

TABLE 4  
MICROCHEMICAL CRYSTALLIZATION OF  
METHADONE HYDROCHLORIDE

<u>Reagent</u>	<u>Concentra- tion of Methadone HCl</u>	<u>Reaction</u>
Potassium iodide, 5%	1:1,000	Colorless crystals
Potassium ferrocyanide, 5% (fresh solution)	1:500	Colorless crystals
Potassium ferricyanide, 5% (fresh solution)	1:500	Yellow crystals
Marme's reagent (fresh solution)	1:10,000	Colorless crystals
Mayer's reagent	1:20,000	Colorless crystals
Wagner's reagent	1:1,000	Pale brown crystals
Lanthanum nitrate, 20%	1:20	Colorless crystals

crystal violet<sup>3,5</sup> or methyl violet,<sup>6</sup> has been described in detail. Each 1 ml. of 0.1 N perchloric acid is equivalent to 34.59 mg. of  $C_{21}H_{27}NO \cdot HCl$ . Potentiometric end point detection has also been used.<sup>99</sup>

### 8.2.2 Direct Titration

Johnson and King<sup>100</sup> developed a rapid direct titrimetric method, using an extractive end point, for determination of methadone in pharmaceutical preparations. Chloroform is added to the organic base dissolved in pH 2.8 acetate buffer solution so that the ratio of chloroform to the aqueous phase is about 3 to 1. Titration is performed using sodium dicotylsulfosuccinate and Dimethyl Yellow screened with Oracet Blue as indicator. The change of color of the chloroform phase from green to pink indicates the end point. The accuracy of the method is  $\pm 1\%$ .

### 8.3 Chloride Determination (Mercuric Nitrate Titration)<sup>2</sup>

A sample of methadone hydrochloride containing at least 2 mg. of chlorine is dissolved in 80 ml. of water-methanol (20:60) mixture and three drops of diphenyl carbazone solution (5 mg./ml. methanol) are added. The sample solution is then titrated with standard 0.5 N mercuric nitrate solution to the first sign of rose color, using a one-ml. microburette.

percent chlorine =

$$\frac{\text{ml. mercuric nitrate} \times \text{normality} \times 35.5 \times 100}{\text{mg. sample}}$$

percent purity of methadone hydrochloride =

$$\frac{\text{percent chlorine-found} \times 100}{\text{percent chlorine-theory}}$$



#### 8.4 Ultraviolet Analysis

Wallace et al.<sup>101</sup> oxidized methadone with barium peroxide to benzophenone which was extracted in heptane and measured spectrophotometrically at 247 nm.,  $\epsilon = 18,713$ . This represents an increase in molar absorbance of approximately 34 times over that of methadone in 0.1 N hydrochloric acid,  $\epsilon = 554$ , at 292 nm. The method is successfully used for determining methadone in biological specimens, namely urine, liver, lungs, kidneys, stomach, and intestines.

#### 8.5 Fluorometric Analysis

The formation of a fluorophore when methadone is heated in a solution of formaldehyde and concentrated sulfuric acid followed by addition of water is reported by McGonigle.<sup>102</sup> The fluorescence is recorded at an excitation wavelength of 270 nm. and an emission wavelength of 450 nm. The aqueous fluorophore is stable for at least 1.5 hours at room temperature. This procedure is suitable for measuring microgram quantities of methadone. Morphine, heroin, codeine, and cocaine do not interfere. However, prior separation of amphetamine, meperidine, and quinine is required since they fluoresce under the conditions of the assay and interfere with the determination of methadone. The relative standard deviation of the methadone assay is 2.2%.

Application of fluorescence and gas chromatography to mass drug screening is presented by Santinga.<sup>103</sup> The smallest detectable quantity of methadone is 1  $\mu\text{g.}/\text{ml}$ . Lawler et al.<sup>104</sup> used spectrophotofluorometry for the determination of methadone in tissues.

#### 8.6 Infrared Analysis<sup>105</sup>

The sample of methadone hydrochloride solution is made alkaline with (1:1) sodium hydroxide in water. The precipitated base is extracted with chloroform. The extracts are dried over anhydrous sodium sulfate and the chloroform is evaporated using steam heat and

a current of air. The residue is dissolved in chloroform and the absorbance, versus a chloroform blank, is determined at  $5.87\ \mu$  using 0.1 mm. cells and a Beckman IR spectrophotometer. The absorbance of the standard is determined in the same manner and the mg. methadone hydrochloride is calculated from the relative ratio. Ultraviolet irradiation of a methanolic solution of methadone hydrochloride, for 1.5 hours, causes 74% deterioration as shown by this method.

### 8.7 Colorimetric Analysis

Drugs containing basic groups form a colored complex with sulfonic acid indicator dyes such as Methyl Orange and Bromcresol Green.<sup>106</sup> The colored complex is then separated from the excess dye by extraction into chloroform or a suitable organic solvent and the quantity of the complexed drug is estimated spectrophotometrically.

The methadone-dye complex is formed using bromthymol blue,<sup>83</sup> bromcresol green,<sup>84</sup> bromphenol blue,<sup>84</sup> bromchlorophenol blue,<sup>84</sup> chlorophenol red,<sup>84</sup> bromcresol purple,<sup>84,107</sup> and methyl orange.<sup>62,82</sup> With these dyes, any basic amine which can form an organic solvent-soluble dye complex would react as methadone, hence precautions need to be taken to eliminate interfering substances. For the determination of methadone in tissues, Rickards et al.<sup>60</sup> liberated the compound by disintegration of the tissue with strong alkali, ether extraction, nitration of the phenyl radicals in methadone, color development with ethyl methyl ketone and measuring the color at 565 nm. The reproducibility and sensitivity are  $\pm 5\%$  and  $1\ \mu\text{g.}$  of methadone. However, it is to be noticed that any methadone metabolic fragment retaining the phenyl and amine groups would react as the parent substance.<sup>81</sup> Nitration of methadone with a mixture of  $\text{HNO}_3/\text{H}_2\text{SO}_4$  (1:1) and the subsequent photometric determination of the

colored derivative using filter S42 (428 nm.) is presented by Skora.<sup>108</sup> The method is used for determination of methadone in ampoules, tablets, and biological media (blood, liver and urine).

An automated method for the determination of methadone hydrochloride in dissolution samples was developed by Bechtel and Brickley.<sup>109</sup> The dissolution samples are sequentially sampled by an AutoAnalyzer and injected into an air-segmented stream of pH 1.2 buffer (U.S.P.) which is pumped through a glass mixing coil. Bromcresol purple (BCP) dye solution is then added to the stream of the methadone sample and the solutions are mixed in a Teflon coil for the formation of the dye-complex. The methadone-dye complex is extracted into ethylene dichloride and the color intensity is measured at 420 nm. The relative standard deviation (R.S.D.) and the relative error (R.E.) are  $\pm 1.48\%$  and  $+ 0.05\%$  respectively.

The specific assay of basic drugs in urine by CM-cellulose column chromatography using continuous drug-dye complex extraction as a detection system is described by McMartin et al.<sup>106</sup>

### 8.8 Polarography

The keto group of methadone is not polarographically reducible because the double bonds are not conjugated. Therefore, an electroactive nitro derivative is prepared. Skora<sup>108</sup> nitrated methadone using a mixture of nitric acid/sulfuric acid (1:1). Nitration is completed in 30 minutes after heating on a boiling water bath. The mixture is then cooled, diluted with 5 ml. distilled water and made alkaline, pH 10, with 5 N NaOH. Three drops of 0.5% gelatin solution are added and the solution is polarographed, after deoxygenation, in a potential range of -0.4 to -1.2 V. The half-wave potential of the nitrated methadone is -0.64 V relative to a saturated calomel electrode. A straight line is obtained when the concentration of the nitrated methadone

derivative is plotted versus the diffusion current (height of the reduction wave) for concentrations of 20, 30, 40, 50, and 60  $\mu\text{g./ml.}$  Concentrations lower than 5  $\mu\text{g./ml.}$  can be determined. The reduction wave of methadone nitrate is distinct and this method is used for assaying methadone in ampoules and tablets. Application of the method to assay for the drug in blood, liver and urine is discussed.

Cathode ray polarography of methadone N-oxide in Walpole's acetate buffer pH 5 gives a reduction wave which has a peak potential of -1.21 V. Reduction of the same solution with  $\text{TiCl}_3/\text{HCl}$  gives methadone.<sup>74</sup>

### 8.9 Bioassay

Schaumann<sup>63</sup> reported a bioassay procedure for methadone using isolated guinea-pig gut. The method is sensitive to methadone at a concentration as low as  $10^{-8}$  M.

### 8.10 Spin Immunoassay

Free-radical technology is combined with immunoassay to yield the new method of "spin immunoassay" used for detection and assay of small molecules in biological fluids.<sup>110-112</sup> An antibody is made against the haptene to be assayed. The antigen is first prepared by coupling the haptene to bovine serum albumin and immunizing rabbits or goats. Ammonium sulfate is used to precipitate the  $\gamma$ -globulin fraction of the serum. The haptene is spin labeled using a stable nitroxide radical. The spin of the unpaired electron of the labeled compound produces a magnetic moment which is detected and measured in an electron spin resonance (ESR) spectrometer. Very broad spectral peaks are observed when the complex of spin-labeled haptene with antibody in a capillary tube is placed in the cavity of the ESR spectrometer. This reflects the immobilization of the free radical at or near the antibody site. Sharp peaks result from the displacement of the spin-labeled haptene by free haptene (as by methadone

in urine or saliva). The amplitudes of the sharp peaks measure quantitatively the number of the free radical molecules tumbling freely in solution and is a direct measure of the haptene concentration. A concentration greater than  $5 \times 10^{-4}$  M of methadone hydrochloride or methadone cyclic metabolites is required to produce spin immunoassay responses equivalent to  $0.5 \mu\text{g./ml.}$  ( $1.8 \times 10^{-6}$  M) of morphine. The advantages, disadvantages and comparison of this technique to thin layer chromatography are discussed by Leute et al.<sup>110</sup>

#### 8.11 Radiotracer Techniques

<sup>14</sup>C and <sup>3</sup>H are used to label methadone. The <sup>14</sup>C activity is assayed as a thin layer of barium carbonate mounted on aluminum discs and counted under a bell counter using a thin mica window<sup>59</sup> and a Geiger-Muller counter.<sup>113</sup> In recent years, liquid scintillation counters have been used for determining the radioactivity of <sup>14</sup>C and <sup>3</sup>H labeled samples.<sup>10,66,114-116</sup>

#### 8.12 Column Chromatography

Application of CM-cellulose column chromatography followed by continuous dye complex extraction is used for the assay of methadone in human urine.<sup>106</sup>

Samples containing methadone are introduced on Amberlite XAD-2 resin column and eluted with methanol,<sup>79,117,118</sup> methanol-ammonia (300:5),<sup>10</sup> chloroform-isopropanol (3:1),<sup>119</sup> and water<sup>80</sup> prior to further analysis. The XAD-2 resin is a styrene-divinylbenzene copolymer and has the capability of adsorbing many water-soluble organic compounds principally by Van der Waal forces.<sup>119</sup>

The effectiveness of recently developed resins BRX-SM-1, -2, -4, and Porapak type Q are compared with XAD-2 resin by Bastos et al.<sup>120</sup> The recovery of <sup>3</sup>H-methadone is somewhat similar for each resin and ranges from 45.4 to 56.0%.

Methadone is resolved from opiates and diluents in illicit narcotic mixtures by using a column of SE-Sephadex C-25 ion-exchanger.<sup>121</sup>

Disposable chromatographic columns are used to extract methadone from urine.<sup>122</sup>

#### 8.13 Paper Chromatography

Paper chromatography on buffered Whatman No. 1 is used by Axelrod<sup>90</sup> to prove the N-demethylation of methadone.

Table 5 summarizes the paper chromatography systems for methadone.

#### 8.14 Thin Layer Chromatography (TLC)

A comparison of the thin layer chromatography of methadone on seven commercially available silica gel coated films and sheets with silica gel coated glass plates using chloroform/n-butanol/ammonium hydroxide (70:40:5), and benzene/dioxane/ethanol/ammonium hydroxide (50:50:5:5) is presented by Schweda.<sup>129</sup> The hand-coated silica gel layer on the glass plates is the most vulnerable layer. The films are superior to it. The silica gel sheets require careful handling. Ho et al.<sup>130</sup> use mini thin layer plates (3 x 3 cm.) to detect the presence of methadone, 100 µg./ml., in an unhydrolyzed urine sample. The developing time is usually 1.5 min. Copenhaver and Blose<sup>131</sup> use slides to prepare thin layer microplates for fast detection of methadone and other drugs of abuse in urine. Gupta<sup>132</sup> uses disposable plastic bags to run the thin layer chromatography of methadone, methadol, normethadol, and acetylmethadol. Identical results are obtained from plates developed in the plastic bag or in a glass tank. Dole et al.<sup>133-135</sup> use ion-exchange paper extraction prior to TLC. Two-dimensional TLC is used for identification of the metabolites of methadone.<sup>80</sup> Fisher et al.<sup>136</sup> chromatograph methadone on precoated, flexible thin layer sheets. The sensitivity of TLC for detection of methadone is discussed by Gorodetzky.<sup>137</sup>

TABLE 5

## PAPER CHROMATOGRAPHY SYSTEMS FOR METHADONE

<u>Solvent System</u>	<u>Paper</u>	<u>Detection*</u>	<u>R<sub>f</sub></u> <u>(× 100)</u>	<u>Reference</u>
tert.-Amyl alcohol/ n-butyl ether/water (80:7:13)	Schleicher & Schüll, #591-C, pH 3.0 pH 4.0 pH 5.0 pH 6.0	IP	35 49 22 56	123
410 Butanol/formic acid/ water (12:1:7)	Schleicher & Schüll, #2045b	BCG	78	124
Dichloroethane/ glacial acetic acid/ water (20:8:2)			75	91, 124
n-Butanol/citric acid/ water (50:1:50) <sup>1</sup>	Whatman No. 1 dipped in 5% sodium hydrogen citrate	D	74	125

(continued ...)

TABLE 5 (concluded)

<u>Solvent System</u>	<u>Paper</u>	<u>Detection*</u>	<u>R<sub>f</sub></u> <u>(× 100)</u>	<u>Reference</u>
Acetate buffer, <sup>2</sup> pH 1.00	Whatman No. 3 impregnated with tributyrin (10% v/v in acetone)	IP	86° 93	126
			95° 88	126
pH 3.30			86° 76	126
pH 4.58			86° 75	126
			95° 67	126
			95° 59	128
pH 7.40			86° 1	126
			95° 2	126
M/15 Phosphate buffer, pH 7.40			86° 0	128

---

<sup>1</sup>Upper layer was used

<sup>2</sup>According to Vogel<sup>127</sup>

**\*Key-** BCG: bromcresol green, D: Dragendorff, IP: iodoplatinate



TABLE 6

## THIN LAYER CHROMATOGRAPHY SYSTEMS FOR METHADONE

<u>Solvent System</u>	<u>Adsorbent*</u>	<u>Detection*</u>	<u>R<sub>f</sub></u> <u>(x 100)</u>	<u>Reference</u>
Benzene/ethyl acetate/ methanol/ammonium hydroxide (80:20:1.2:0.1)	SG	RS	58	10
	SG	IP	68	123
Ethyl acetate/methanol/ ammonium hydroxide (85:10:5)	SG	DPNA	95	138
	SG	IP	96	117
	SG	IP	65	118
	SG	IP	91	130
	SG	IP	96	133
	SG	IP	77	134, 135
	SG	IP	82	136
	SG	IP	96.9	137
	SG	IP	99	139
	SG	BCG	84	140
Dioxane/ethanol/pyridine/ water (25:50:20:5)	C	IP	34	15
Ethanol/glacial acetic acid/ water (60:30:10)	C	IP	59	15
	SG	D	46	76

(continued ...)

TABLE 6 (Continued)

<u>Solvent System</u>	<u>Adsorbent*</u>	<u>Detection*</u>	<u>R<sub>f</sub></u> <u>(× 100)</u>	<u>Reference</u>
Benzene/dioxane/ethanol/ ammonium hydroxide (50:40:5:5)	C	IP	99	15
	SG	D	75	76
Benzene/n-butanol/methanol/ water (10:15:60:15)	C	IP	17	15
	SG	D	15	76
tert.-Amyl alcohol/n-butyl ether/water (80:7:13)	SG	IP	17	76
n-Butanol/glacial acetic acid/ water (4:1:2)	SG	IP	55	76
	SG	IP	64	141
n-Butanol/conc. hydrochloric acid, saturated with water (90:10)	C	IP	62	15
	SG	IP	57	141
Ethyl acetate/methanol/ ammonium hydroxide (85:10:3)	SG	IP	96	142
Ethanol/hexane (8:92)	SG	IP	31	142
Ethyl acetate/methanol/ ammonium hydroxide (85:10:1)	SG	IP + D	94	143

(continued ...)

TABLE 6 (Continued)

<u>Solvent System</u>	<u>Adsorbent*</u>	<u>Detection*</u>	<u>R<sub>f</sub></u> <u>(x 100)</u>	<u>Reference</u>
Carbon tetrachloride	SG	IP	11	144 <sup>1</sup>
Isopropyl ether	SG	IP	27	144 <sup>1</sup>
Benzene	SG	IP	42	144 <sup>1</sup>
Ethylene dichloride	SG	IP	37	144 <sup>1</sup>
Methylene chloride	SG	IP	58	144 <sup>1</sup>
Chloroform	SG	IP	55	144 <sup>1</sup>
Ethyl ether	SG	IP	67	144 <sup>1</sup>
Ethyl acetate	SG	IP	73	144 <sup>1</sup>
n-Butyl alcohol	SG	IP	61	144 <sup>1</sup>
Isopropyl alcohol	SG	IP	63	144 <sup>1</sup>
Acetone	SG	IP	79	144 <sup>1</sup>
Methanol	SG	IP	77	144 <sup>1</sup>

(continued ...)

TABLE 6 (Continued)

<u>Solvent System</u>	<u>Adsorbent *</u>	<u>Detection *</u>	<u>R<sub>f</sub> (× 100)</u>	<u>Reference</u>
Ethyl acetate/methanol/ ammonium hydroxide (85:10:10)	SG	IP	95	145
Ethyl acetate/methanol/water/ ammonium hydroxide (85:10:3:1)	SG	SUV	80	145
Methanol/ammonium hydroxide (99:1)	SG	I	83	145
	SG	IP	48	146
Chloroform/methanol/ammonium hydroxide (90:10:1)	SG	IP	80	145
Acetic acid/butanol/water (1:4:5) <sup>2</sup>	SG	IP	50	147
Chloroform/methanol (4:1)	SG	IP	48	95
Ethyl acetate/methanol/ ammonium hydroxide (85:10:2.5)	--	IP	73	148
Ethyl acetate/methanol (85:10)	SG	IP	68	149 <sup>1</sup>
Chloroform/methanol (80:20)	SG	IP	69	149 <sup>1</sup>

(continued ...)

TABLE 6 (Continued)

<u>Solvent System</u>	<u>Adsorbent*</u>	<u>Detection*</u>	<u>R<sub>f</sub></u> <u>(× 100)</u>	<u>Reference</u>
Ethyl acetate/cyclohexane/ p-dioxane/methanol/water/ ammonium hydroxide (50:50:10:10:1.5:0.5)	SG	SA	83	150
Ethyl acetate/cyclohexane/ p-dioxane/methanol/water/ ammonium hydroxide (50:50:10:10:0.5:1.5)	SG	IP	91	150
Ethyl acetate/cyclohexane/ methanol/water/ammonium hydroxide (70:15:8:0.5:2)	SG	ASA	94	150
Ethyl acetate/cyclohexane/ ammonium hydroxide (50:40:0.1)	SG	BCG	98	150
Benzene/cyclohexane/ diethylamine (15:75:10)	SG	IP	99	123
Ethyl acetate/dimethylformamide (3:1)	SG	IP	99	123
tert.-Amyl alcohol/n-butyl ether/water (14:7:1)	SG	IP	55	123

(continued ...)

TABLE 6 (Continued)

<u>Solvent System</u>	<u>Adsorbent</u> *	<u>Detection</u> *	$R_f$ ( $\times 100$ )	<u>Reference</u>
tert.-Amyl alcohol/n-butyl ether/water (80:7:13)	SG	IP	86	123
Chloroform/methanol/ammonium hydroxide (85:10:1)	SG <sup>3</sup>	IP	80	151
Ethyl acetate/methanol/ammonium hydroxide (85:10:1.5)	SG <sup>3</sup>	IP	67	151
Chloroform/methanol (9:1)	SG	IP	17	146
	SG	IP	32	152
Acetone	SG	IP	20	146
Acetone/ammonium hydroxide (99:1)	SG	IP	59	146
Methanol	SG	IP	16	146
Chloroform/methanol (50:50)	SG	IP	20	146
Chloroform/methanol/ammonium hydroxide (47.5:47.5:5)	SG	IP	80	146
Chloroform/glacial acetic acid/methanol (47.5:5:47.5)	SG	IP	54	146

(continued ...)

TABLE 6 (Continued)

<u>Solvent System</u>	<u>Adsorbent*</u>	<u>Detection*</u>	<u>R<sub>f</sub> (× 100)</u>	<u>Reference</u>
Benzene/dioxane/ethanol/ ammonium hydroxide (50:40:5:5) <sup>2</sup>	SG	IP	98	153
Methanol/12 N ammonium hydroxide (100:1.5)	SG	IP	42	153
	SG	IP	53	152
	SG	IP	37	128
	SG	D + IP	97	154
n-Butanol/ethyl acetate/ ethanol/ammonium hydroxide (2:28:14:0.4)	SG	IP	71	133
Benzene/ether (10:1)	A	IP	20	155
Dichloromethane/ether (10:2)	A	IP	35	155
Benzene/diethylamine/dioxane/ ethanol (50:5:40:5)	SG	IP	91	156
Dimethylformamide/ethyl acetate (1:3)	SG	IP	86	157
Ethanol/isopropyl ether (20:80)	SG	IP	11	152

(continued ...)

TABLE 6 (Continued)

<u>Solvent System</u>	<u>Adsorbent*</u>	<u>Detection*</u>	<u>R<sub>f</sub> (× 100)</u>	<u>Reference</u>
Acetoacetic ester/chloroform (1:1)	CC	SUV + D	88	158
Acetone/chloroform/formic acid (4:16:1)	A	D	59	159
Cyclohexane/diethylamine (9:1)	SG	D	71	159
Benzene/chloroform/ diethylamine (6:3:1)	SG	D	88	159
Benzene/1,4-dioxane/ethanol/ ammonium hydroxide (100:80:10:11)	SG	D + IP	97	154
Acetic acid/chloroform/ methanol (10:35:65)	SG	SUV	53	160
Benzene/ethyl acetate/ ammonium hydroxide (35:60:5)	SG	SUV	75	160
Acetone/chloroform/ diethylamine (2:88:10)	SG	D	72	92

(continued ...)



TABLE 6 (Continued)

<u>Solvent System</u>	<u>Adsorbent*</u>	<u>Detection*</u>	<u>R<sub>f</sub> (× 100)</u>	<u>Reference</u>
Benzene/diethylamine/methanol (75:10:15)	SG	D	74	92
Benzene/n-butanol/methanol/ water/ammonium hydroxide (10:15:60:10:5)	SG	D	79	92
Ethanol/ethyl acetate/ ammonium hydroxide (50:45:5)	SG	D	73	76
Chloroform/dioxane/ethyl acetate/ammonium hydroxide (25:60:10:5)	SG	I, D, PP	73	161

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<sup>1</sup>In an ammonium hydroxide atmosphere

<sup>2</sup>Top layer was used

<sup>3</sup>Silica gel GF with 10%  $\text{CaSO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$

\*Key - A: alumina, ASA: ammonical silver nitrate and heat, BCG: bromcresol green, C: cellulose, D: Dragendorff, DPNA: diazotized  
(continued ...)

TABLE 6 (Concluded)

p-nitroaniline, I: 1% iodine, IP: iodoplatinate, PP: 0.1%  
potassium permanganate, RS: radioscanning, SA: 0.5% sulfuric acid,  
SG: silica gel, SUV: short ultraviolet light

Table 6 contains the most commonly used thin layer chromatography systems for methadone.

#### 8.15 Gas Chromatography (GC)

Gas chromatography systems for methadone are reported in Table 7. A flame ionization detector is used in all references unless otherwise indicated.

#### 8.16 Combined Gas Chromatography-Mass Spectroscopy (GCMS)

The GCMS conditions and the mass fragmentation pattern have been previously discussed (See 2.14). This technique is used for the identification of methadone metabolites<sup>59,74,77-80</sup> and for the screening and identification of the dangerous drugs of abuse.<sup>171,172</sup>

#### 8.17 High Pressure Liquid Chromatography (HPLC)

High pressure liquid chromatography is used by Lorenz<sup>173</sup> for the determination of isomethadone in methadone. A reverse phase system is used. The column, 1 meter long x 2 mm. i.d. is packed with DuPont Permaphase ETH packing material. The mobile liquid consists of 1% reagent ammonium hydroxide, 15% methanol, and 84% water. The column is operated at a flow rate of 50 ml./hr. A 254 nm. detector is used for monitoring the column eluent. The retention volumes to elute isomethadone and methadone are 6 ml. and 17 ml. respectively.

### 9. Extraction from Biological Fluids

Most methods used for the determination of drugs in biological fluids involve three steps, namely solvent extraction, concentration, and detection or assaying. Each of these steps is time-consuming and drug losses due to adsorption on to glassware, incomplete transfer of solvents and evaporation of volatile compounds may lower the recovery and hence the sensitivity. Ramsey and Campbell<sup>163</sup> described

TABLE 7  
GAS CHROMATOGRAPHY SYSTEMS FOR METHADONE

<u>Column</u>	<u>Carrier Gas</u>	<u>Flow Rate ml./min.</u>	<u>Column Temp. °C.</u>	<u>Retention Time, min.</u>	<u>Reference</u>
3 ft. long × 0.125 in. O.D., 3% OV-17 on 100/120 mesh Gas Chrom Q	N <sub>2</sub>	30	190	7.40	68
6 ft. long × 3 mm. I.D., 2% SE-30 on 80/100 mesh Gas Chrom S	A	47	215	3.32	15
6 ft. long × 2 mm. I.D., 3% SE-30 on 80/100 mesh Gas Chrom Q	He	32	200	2.80	162
2 M long × 0.125 in. O.D., 2.5% E-301 on 80/100 mesh Chromosorb G	N <sub>2</sub>	30	200	5.20	163
4.75 ft. long × 0.128 in. diameter, SE-30 on 70/80 mesh DMCS	-	-	-	14.8	149
1.22 M long × 5.5 mm. I.D., 3% OV-17 on 100/200 mesh Gas Chrom Q	N <sub>2</sub>	50	240	7.7	164

(continued ...)

TABLE 7 (Continued)

<u>Column</u>	<u>Carrier Gas</u>	<u>Flow Rate ml./min.</u>	<u>Column Temp. °C.</u>	<u>Retention Time, min.</u>	<u>Reference</u>
3% OV-1	-	-	220	2.6	165
3% OV-17	-	-	220	1.7	165
3% OV-210	-	-	220	1.8	165
5 ft. long × 0.125 O.D., 5% SE-30 on 60/80 mesh Chromosorb W	N <sub>2</sub>	30.7	230	4.7	166
6 ft. long × 4 mm. I.D., 1% SE-30 on 100/200 mesh Anakrom ABS	A	65	180	12.1	167 <sup>1</sup>
	A	56	200	4.9	167
	A	70	210	3.3	167
2% OV-225 on Gas Chrom Q	N <sub>2</sub>	35	190	2	153
1.2 M long × 3 mm. I.D., 3.5% UCW98 on 80/100 mesh Chromosorb W-AW-DMCS	N <sub>2</sub>	35	235	1.2	168
1.2 M long × 4 mm. I.D., 3% SE-30 on 80/100 mesh Gas Chrom Q	He	75	210	4	160

(continued ...)

TABLE 7 (Continued)

<u>Column</u>	<u>Carrier Gas</u>	<u>Flow Rate ml./min.</u>	<u>Column Temp. °C.</u>	<u>Retention Time, min.</u>	<u>Reference</u>
4 ft. long × 2.5 mm. I.D., 1% W-98 on 80/100 mesh Gas Chrom Q	He	60	165	4	57, 78
2 M long × 0.25 in. O.D., 3% OV-17 on 60/80 mesh acid washed, DMCS treated Gas Chrom Q	N <sub>2</sub>	65	195	12	92
1 M long × 0.125 in. O.D., 2% Carbowax on 80/100 mesh acid washed, DMCS treated Chromosorb G	N <sub>2</sub>	36	180	12	92
6 ft. long × 0.25 in., 5% OV-1 on 100/120 mesh Chromosorb W	N <sub>2</sub>	50	235	3.3	9
1.3 M long × 6.25 mm. O.D., 3.8% UC-W98 on Diatoport S	-	-	190	6.4	77
2 M long × 0.25 in. O.D., 2% SE-30 on 80/100 mesh Chromosorb G	N <sub>2</sub>	16	180	14.2	76

(continued ...)

TABLE 7 (Continued)

<u>Column</u>	<u>Carrier Gas</u>	<u>Flow Rate ml./min.</u>	<u>Column Temp. °C.</u>	<u>Retention Time, min.</u>	<u>Reference</u>
1 M long × 0.125 in. O.D., 5% KOH and 2% Carbowax 20 M	N <sub>2</sub>	14	185	7.9	76
4 ft. long × 3 mm. I.D., 3.8% W98 on 80/100 mesh Diatoport S	N <sub>2</sub>	80	175	5.4	68
6 ft. long × 3 mm. I.D., 1% cyclohexane dimethanol succinate on 100/120 mesh Diatomite CQ	N <sub>2</sub>	80	185	4.6	68
2 ft. long × 4 mm. I.D., 2.5% SE-30 on 80/100 mesh Chromosorb G	N <sub>2</sub>	40	200	1.7	169
6 ft. long × 0.25 in. I.D., 3% OV-1 on 80/100 mesh Chromosorb WHP	N <sub>2</sub>	60	255	1.3	170

(continued ...)

TABLE 7 (Concluded)

<u>Column</u>	<u>Carrier Gas</u>	<u>Flow Rate ml./min.</u>	<u>Column Temp. °C.</u>	<u>Retention Time, min.</u>	<u>Reference</u>
6 ft. long × 0.25 in. O.D., 3% SE-30 on 100/ 200 mesh Gas Chrom Q	N <sub>2</sub>	40	205	5.25	145

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<sup>1</sup>Strontium 90 argon ionization detector



a rapid method for extraction of methadone in which the complete analysis is carried out in one vessel and omitting the evaporation step, to overcome the volatility problems. Lyophilization and liquid-solid extraction are used to detect drugs of abuse in urine.<sup>142</sup>

Reliability of identification techniques for drugs of abuse in a urine screening program and drug excretion data is presented by Kaistha and Jaffe.<sup>174</sup> The drug is absorbed on cation-exchange resin loaded paper and the ion paper is extracted at pH 1 with  $\text{CHCl}_3$  and then chromatographed on thin layer plates. The concentration of methadone in the urine by ion exchange paper provides a cleaner extract than by direct extraction of the urine.<sup>147</sup> Dole et al. describe buffer elution of the ion exchange paper<sup>135</sup> and the application of Amberlite IR-120 cation exchange paper prior to thin layer chromatography.<sup>134</sup> Comparison of three urine extraction techniques is reported by Kaistha and Jaffe.<sup>175</sup>

"Clean-up" procedures for urine and blood for the determination of the drugs of abuse are reviewed by Sohn et al.<sup>176</sup>

Kaistha et al.<sup>177</sup> recently evaluated the drug abuse screening programs, detection procedures, development costs, street-sample analyses and field tests.

#### 10. Determination in Tissues

The previous methods emphasize the determination of methadone in blood samples, urine samples and urine screens. However, Lawler et al.<sup>104</sup> survey the quantitative assays of methadone in tissues. The drug in the tissue is examined by spectrophotofluorometry following formaldehyde treatment and using excitation at 270 nm. and emission at 450 nm. Gas liquid chromatography is also used, after silylation, on a 3% OV-17 column packed on 100/120 mesh Gas Chrom Q and heated at 220°C.

## 11. Bibliography

A comprehensive bibliography of "Methadone, 1929-1971" has been published in two parts.<sup>178,179</sup> Langrod<sup>180</sup> compiled a bibliography of methadone maintenance treatment of heroin addiction. The reader is referred to these three lists of references for complete literature about methadone.

The literature search for preparing this profile was conducted through April of 1973.

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## **OXAZEPAM**

*Charles M. Shearer and Caesar R. Pilla*

CONTENTS

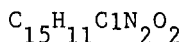
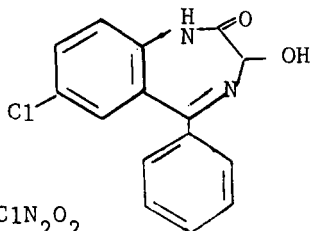
1. Description
  - 1.1 Name, Formula, Molecular Weight
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# OXAZEPAM

## 1. Description

### 1.1 Name, Formula, Molecular Weight

The name used by Chemical Abstracts and the National Formulary XIII for oxazepam is 7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-2H-1,4 benzodiazepin-2-one.



Mol. Wt.: 286.72

### 1.2 Appearance, Color, Odor

Oxazepam is a creamy white to pale yellow powder having practically no odor.

## 2. Physical Properties

### 2.1 Infrared Spectra

An infrared absorption spectrum of a potassium bromide dispersion of oxazepam (NF Reference Standard material) is presented in Figure 1. This spectrum agrees with published spectra<sup>1,2,3</sup>. The spectral band assignments are listed in Table I.

<u>Table I</u>		
<u>Infrared Spectral Assignments of Oxazepam</u>		
<u>Wavelength, <math>\mu</math></u>	<u>Vibration Mode</u>	<u>Reference</u>
3.05 to 3.20	OH, NH stretch	4
5.79 and 5.86	C=O stretch	5
6.19	C=N-stretch	5
6.35 and 6.72	Aromatic C=C deformations	6
12.07	Out of plane CH deformation of 1,2,4 substituted aromatic	6
13.39 and 14.35	Out of plane CH deformation of mono substituted aromatic	6

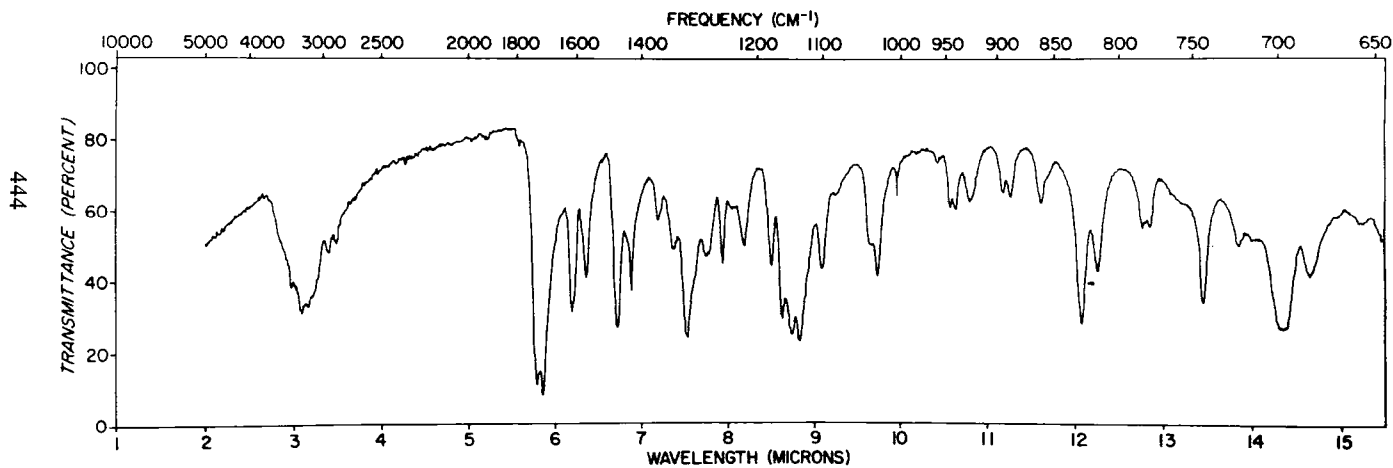


Fig. 1 - I.R. Spectrum of Oxazepam (NF Reference Standard) KBr Pellet



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## 2.2 Nuclear Magnetic Resonance

The 60 MHz NMR spectrum, shown in Figure 2, was obtained by dissolving oxazepam (NF Reference Standard material) in deuterio dimethylsulfoxide containing tetramethylsilane as internal reference. The spectral assignments are listed in Table II.

Table II		
NMR Spectral Assignments of Oxazepam		
Chemical Shift ( $\delta$ )	Protons	Splitting
4.82	aliphatic C-H	Doublet
6.35	O-H	Doublet
7.52	aromatic CH	Multiplet
10.78	N-H	Singlet

Both the hydroxyl and amino protons exchanged with D<sub>2</sub>O. Sadee<sup>8</sup> reported a singlet for the aliphatic C-H proton, however, a singlet was observed in these laboratories only after D<sub>2</sub>O exchange.

## 2.3 Ultraviolet Spectra

The ultraviolet spectra of oxazepam in 0.1N hydrochloric acid, 0.1N sodium hydroxide, and in pH 7 buffer are presented in Figure 3. The spectrum of oxazepam in alcohol is presented in Figure 4. The spectrum and absorptivities of oxazepam in alcohol agree with published values<sup>1,3,9</sup>. The absorptivities and maximum wavelengths are presented in Table III.

Table III		
Ultraviolet Spectral Characteristics		
Solvent	$\lambda$ max (nm)	Absorptivity
0.1N HCl	236	111
	284	41
	362	13
pH 7	230	131
	315	9
0.1N NaOH	236	111
	342	11
alcohol	230	126
	318	9

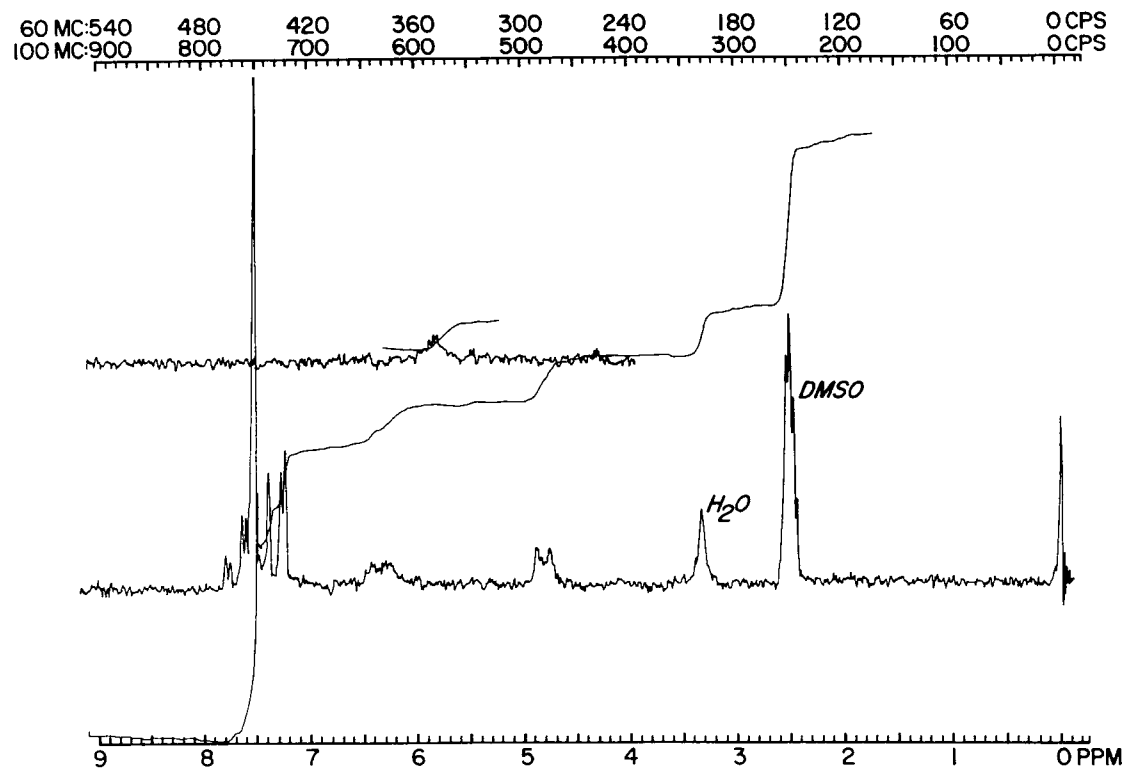


Fig. 2 - NMR Spectrum of Oxazepam (NF Reference Standard) Solvent deuterio dimethylsulfoxide

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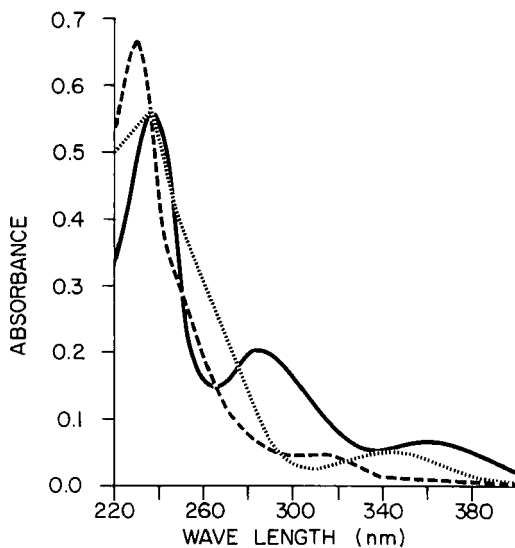


Fig. 3 - Ultraviolet Spectra of Oxazepam (NF Reference Standard) Solvent—0.1N HCl --- pH 7 buffer, ... 0.1N NaOH.

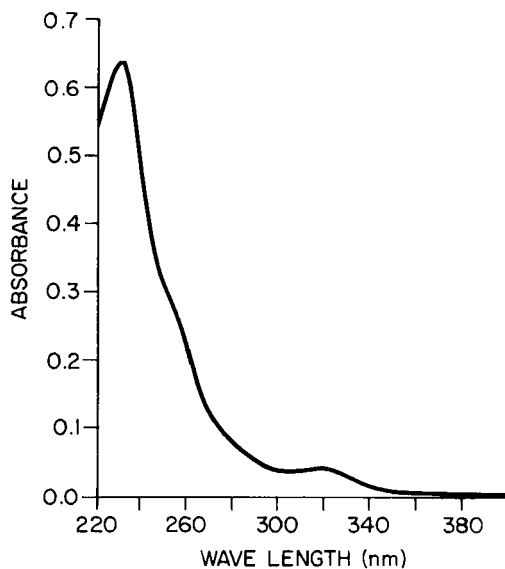


Fig. 4 - Ultraviolet Spectrum of Oxazepam (NF Reference Standard) Solvent-alcohol.

2.4 Mass Spectra

The mass spectrum of oxazepam (NF Reference Standard material) was obtained by direct insertion of the sample into the MS-902 double focusing, high resolution mass spectrometer. The sample was run at 200°C. and  $1.0 \times 10^{-6}$  torr with the ionization electron beam energy at 70 e.v. The high resolution data was compiled and tabulated with the aid of the PDP-8 Digital computer. A line graph of the mass spectrum is shown as Figure 5 and the major high resolution data in Table IV<sup>10</sup>.

Table IV  
Mass Spectrum of Oxazepam

<u>Measured Mass</u>	<u>Calculated Mass</u>	<u>Formula</u>
286.0484	286.0509	$C_{15}H_{11}O_2N_2Cl$
270.0372	270.0321	$C_{15}H_9O_2NC1$
269.0474	269.0481	$C_{15}H_{10}ON_2C1$
268.0436	268.0402	$C_{15}H_9ON_2C1$
267.0319	267.0325	$C_{15}H_8ON_2C1$
259.0427	259.0399	$C_{14}H_{10}O_2NC1$
257.0450	257.0481	$C_{14}H_{10}ON_2C1$
241.0461	241.0420	$C_{15}H_{10}OC1$
239.0379	239.0376	$C_{14}H_8N_2C1$
233.0715	233.0714	$C_{15}H_9ON_2$
229.0526	229.0532	$C_{13}H_{10}N_2C1$
205.0738	205.0765	$C_{14}H_9N_2$
194.0849	194.0844	$C_{13}H_{10}N_2$

This spectrum is in agreement with that presented by Sadee.<sup>11</sup>

The molecular ion was at  $m/e$  286. The base peak,  $m/e$  257, is generated by loss of a formyl radical, which may be preceeded by a hydride migration from C-3 to C-5. Additional loss of CO generated an indazole at  $m/e$  229, which then eliminated Cl to give  $m/e$  194. Other predominant peaks are  $m/e$  269( $M^+ - OH$ ) and  $m/e$  259( $M^+ - CHN$ ).

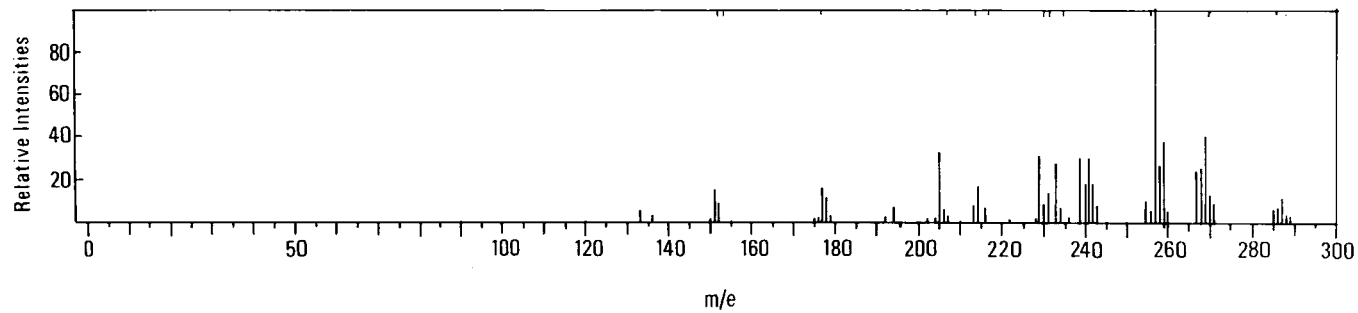


Fig. 5 - Mass Spectrum of Oxazepam (NF Reference Standard)

## 2.5 Melting Range

The following melting point temperatures have been reported:

°C.	Reference
203 - 204	5
205 - 206	12
206	13,14

## 2.6 Differential Thermal Analysis

A study by differential thermal analysis (DTA) reveals that the melting endotherm is a function of the heating rate<sup>15</sup>. This is illustrated in Table V. A DTA curve of oxazepam (NF Reference Standard material) obtained at a heating rate of 10°/min. is included as Figure 6.

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Table V  
Differential Thermal Analysis of Oxazepam

heating rate $\frac{^{\circ}\text{C.}}{\text{min.}}$	melting endotherm °C.
5	188
10	193
20	201

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## 2.7 Solubility

The following solubility data were obtained at uncontrolled room temperature:

4.5 mg./ml. in 95% ethanol

0.03 mg./ml. in water

4 mg./ml. in chloroform

These values agree with the solubilities given in the National Formulary XIII.

## 2.8 Crystal Properties

The X-ray diffraction pattern of oxazepam (NF Reference Standard material) was obtained with a Philips diffractometer using Cu K $\alpha$  radiation<sup>15</sup>. The calculated d spacings of the diffraction pattern are presented in Table VI.

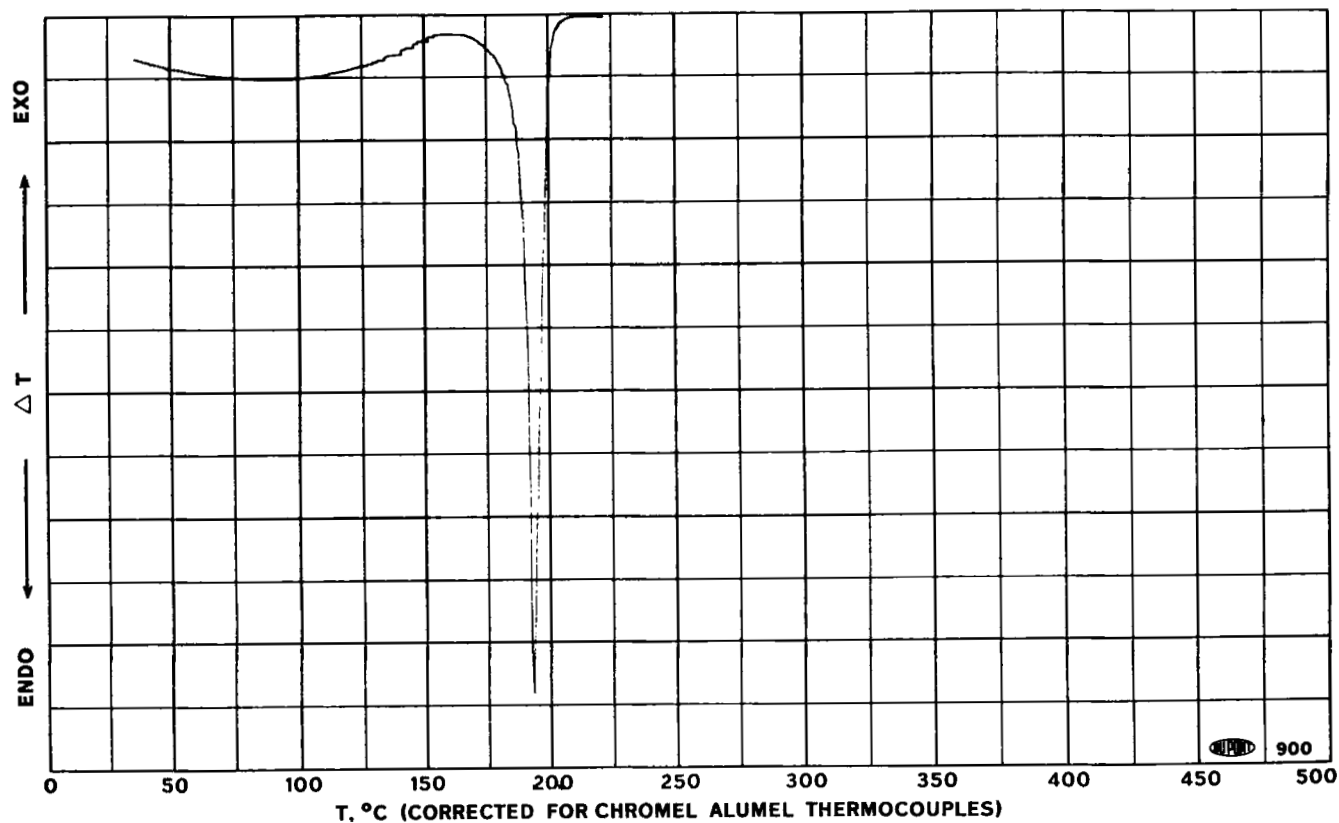


Fig. 6 - DTA Spectrum of Oxazepam (NF Reference Standard)

Table VI  
X-Ray-Powder, Diffraction Pattern  
 Sample: Oxazepam: NF Reference Standard  
 Source: Cu K  $\alpha$

<u>2<math>\theta</math></u>	<u>d</u>	<u>2<math>\theta</math></u>	<u>d</u>	<u>2<math>\theta</math></u>	<u>d</u>
*6.6	13.4	*20.3	4.374	31.0	2.885
12.4	7.13	21.1	4.210	31.4	2.849
13.2	6.70	21.5	4.132	32.2	2.780
14.8	5.98	22.6	3.934	33.3	2.690
16.6	5.34	23.3	3.817	34.0	2.636
17.4	5.096	24.3	3.662	34.4	2.607
17.7	5.010	25.4	3.506	36.8	2.442
18.5	4.796	26.5	3.363	38.7	2.326
19.4	4.576	27.2	3.279	39.0	2.310
*19.8	4.482	29.8	2.998	*Most intense peaks	
20.0	4.440	30.4	2.940		

### 2.9 Dissociation Constant

The pKa's of oxazepam in aqueous solutions were determined spectrophotometrically to be 1.8, at which a proton is gained; and 11.1, at which a proton is lost.

### 3. Synthesis

One synthetic route for the production of oxazepam is given in Figure 7. The quinazoline 3-oxide can be prepared by reacting chloracetyl chloride with 2-amino-5-chlorobenzophenone oxime<sup>16</sup>. The ring expansion step with sodium hydroxide was described by Bell and coworkers<sup>17</sup>. Treatment of the resulting benzodiazepin -2-one 4-oxide with acetic anhydride and hydrolysis of the ester with base yields oxazepam<sup>5</sup>. Other synthetic routes and the chemistry of benzodiazepines in general are discussed in review articles<sup>18,19,20,21</sup>.

### 4. Stability

Oxazepam is stable as a solid or in a neutral solution.<sup>19</sup> Acid hydrolysis produces 2-amino-5-chlorobenzophenone<sup>22</sup>. The imino-carbinol structure leads to a number of rearrangements (Figure 8) upon treatment with acetic acid<sup>5</sup> or base<sup>19</sup>.



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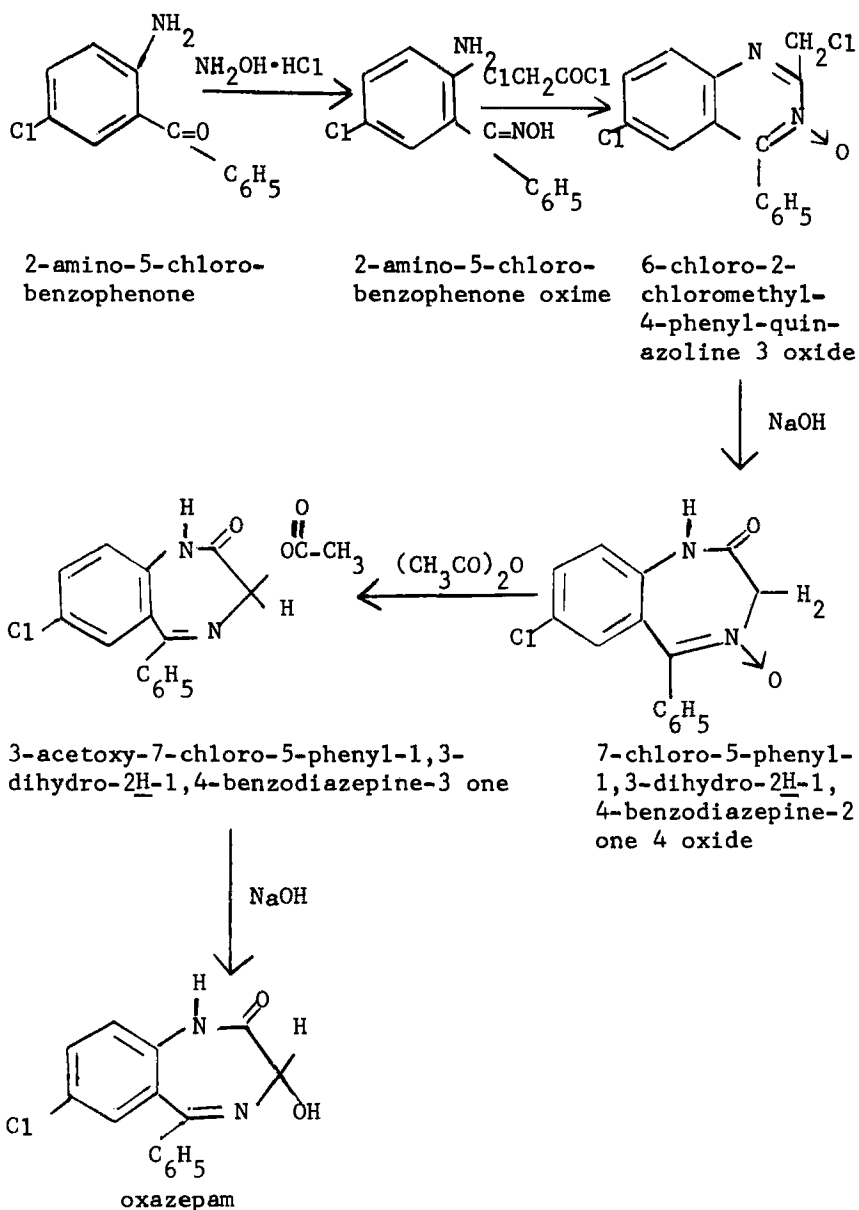


Figure 7 - Synthetic Route for Oxazepam

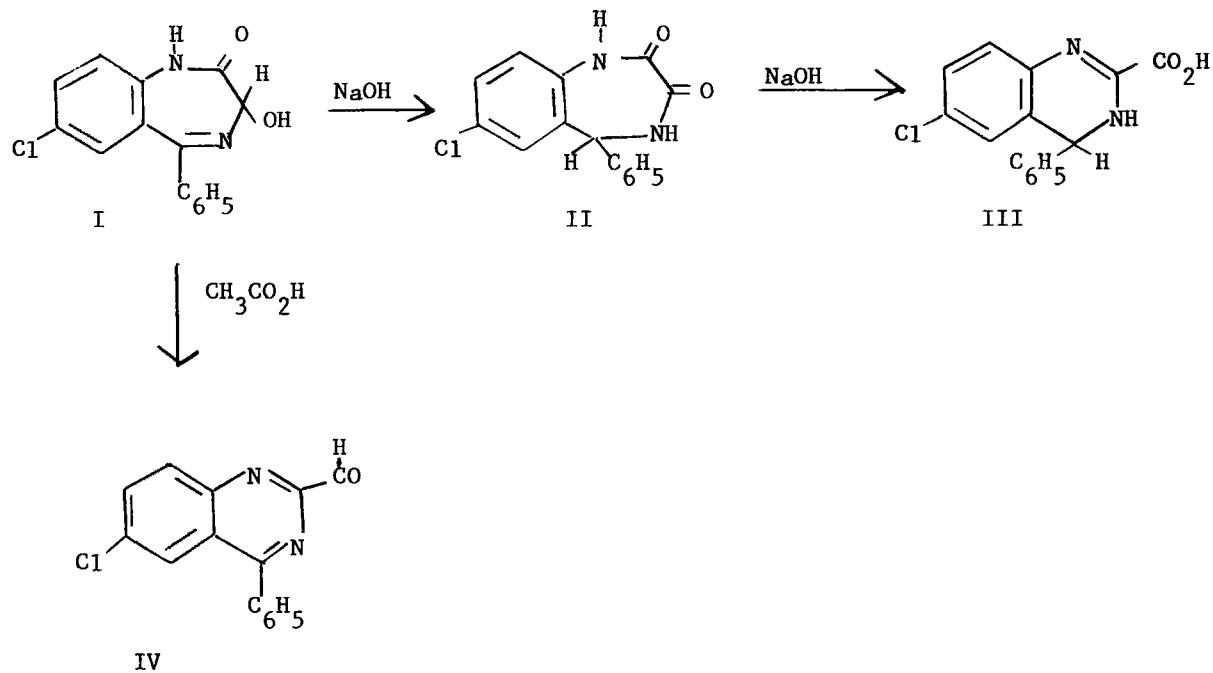


Figure 8 - Degradation of Oxazepam

## 5. Metabolism

The major metabolite (greater than 95%) of oxazepam in man has been determined to be oxazepam glucuronide<sup>22</sup>. Minor metabolites are as follows: 6-chloro-4-phenyl-2(1H)-quinazolinone; 2-amino-5-chlorobenzophenone; 2'-benzoyl-4'-chloro-2,2-dihydroxyacetanilide; 2'-benzoyl-4'-chloro-2-hydroxy-2-ureidoacetanilide; 7-chloro-1,3-dihydro-3-hydroxy-5-(p-hydroxyphenyl)-2H-1,4-benzodiazepin-2-one; and 7-chloro-1,3-dihydro-3-hydroxy-5-[3 (or 4)-hydroxy-4-(or 3) methoxyphenyl]-2H-1,4-benzodiazepin-2-one. The first two minor metabolites exist only in the unconjugated state in urine while the others are present as conjugates as well as in the free form<sup>23</sup>.

## 6. Methods of Analysis

### 6.1 Elemental Analysis

The following data were obtained on NF Reference.

Standard material<sup>7</sup>:

<u>Element</u>	<u>% Theory</u>	<u>% Found</u>
C	62.94	62.71
H	3.85	3.99
N	9.79	9.62
Cl	12.41	12.47

### 6.2 Gravimetric Analysis

Oxazepam can be precipitated out of a very dilute acidic solution with silicotungstic acid. The precipitate is collected, washed with water, dried at 70°C. and weighed. This method has been applied to dosage forms<sup>2</sup>.

When a solution of Reinecke's salt is added to a dilute solution of oxazepam, a bright rose-violet colored precipitate is formed. The precipitate is then washed, dried, and weighed<sup>2,24</sup>.

### 6.3 Direct Spectrophotometric Analysis

Salim and coworkers<sup>1</sup> described an ultraviolet spectrophotometric method of analysis which was applicable to capsules of oxazepam. In this method a sample equiva-

lent to about 50 mg. oxazepam is extracted with alcohol through a sintered glass funnel. This solution is diluted to obtain a final concentration of about 4 mcg./ml. in alcohol. The absorbance is read on a spectrophotometer at 229 nm. using alcohol as a blank and compared with the absorbance of a standard solution of oxazepam. This is also the method specified in the National Formulary XIII.

A completely automated system capable of disintegrating a whole tablet or capsule, dissolving the active constituent, filtering it, diluting a portion of the clear filtrate to a desired volume and obtaining a complete UV-visible scan, has been reported<sup>25</sup>. The accuracy of the automated method is comparable to that of the manual spectrophotometric method for oxazepam.

#### 6.4 Colorimetric Analysis

When a solution of Reinecke's salt is added to a dilute solution of oxazepam a bright rose-violet colored precipitate is formed. This can be isolated and then dissolved in acetone and its concentration determined spectrophotometrically at 525 nm<sup>2,24</sup>.

Oxazepam can be hydrolyzed with hydrochloric acid to form glycine and 2-amino-5-chlorobenzophenone. The aromatic amine can be diazotized with nitrous acid and coupled to naphthylene diamine<sup>26</sup>, N-alpha naphthyl-N-diethyl propylenediamine<sup>27</sup>, N-(1-naphthyl)ethylenediamine<sup>28</sup>, or alpha naphthol<sup>29</sup>. The concentration of the oxazepam in the resulting colored solution can be determined spectrophotometrically in the visible region.

#### 6.5 Fluorometric Analysis

Walkenstein and coworkers<sup>22</sup> applied fluorometry to the determination of oxazepam in biological fluids. Oxazepam was extracted from the body fluids by ethylene dichloride and the fluorescence measured on a fluorometer equipped with a high pressure mercury lamp, a 200-400 mμ broad band primary filter and a B540 (520-660 mμ) broad band secondary filter.

Braun and coworkers<sup>30</sup> developed a fluorometric method for determining oxazepam which had a sensitivity of

0.005  $\mu\text{g.}/\text{ml.}$  In this method an ethanolic solution of oxazepam is heated in phosphoric acid, producing a very intense fluorescence. The fluorescence is measured at an excitation wavelength of 360 nm. and an emission wavelength of 475 nm. This method has also been used for dosage form analysis<sup>31</sup>.

Steidinger and Schmid<sup>32</sup> developed two methods for determining oxazepam, combining thin layer chromatography and fluorometry. In one method, the oxazepam is scraped off the developed TLC plate and extracted with methanol. Perchloric acid is added to this solution and it is heated. The resulting fluorescence can then be determined with a fluorometer. The other method, also reported by Lauffler,<sup>33</sup> is to treat the intact plate with trichloroacetic acid, heat the plate, and determine the fluorescence directly by use of a thin layer chromatographic plate scanner.

#### 6.6 Titrimetric Analysis

Oxazepam can be dissolved in dimethylformamide, and titrated with 0.1N tetrabutylammonium hydroxide [prepared in benzene:methanol (9:1)] to a potentiometric endpoint using glass vs. calomel electrodes<sup>1</sup>.

Beyer and Sadee<sup>34</sup> determined oxazepam by dissolving it in glacial acetic acid and titrating with 0.05N perchloric acid. The endpoint was determined either potentiometrically or visually, using crystal violet as an indicator. Acetic anhydride was also used as a solvent for oxazepam, giving comparable results.

#### 6.7 Polarographic Analysis

The polarographic behavior of oxazepam has been discussed in many papers. Fazzari and Riggleman<sup>35</sup> obtained well-defined cathodic waves at the dropping-mercury electrode in a mixture of methylene chloride-methyl alcohol with a supporting electrolyte of 0.1M tetraethylammonium bromide. The halfwave potential of oxazepam in this system is about -1.02V and the diffusion current is linear with concentration. This method was applied to capsules of oxazepam.

Oelschlager and coworkers<sup>36</sup> also found a linear

relation between concentration and diffusion current in an acetate buffer system containing 20% dimethylformamide. This procedure was used to assay tablets containing oxazepam. They also correlated half-wave potential versus a saturated calomel electrode, with pH as shown below. The pH was determined in a Britton-Robinson buffer containing 20% dimethylformamide.

pH	2.3	3.4	5.1	6.0	7.2	8.2	9.2
$E_{1/2}$ (V)	-.695	-.825	-.955	-1.005	-1.08	-1.125	-1.185

Oeschlager and coworkers<sup>37</sup> further investigated the electrochemistry of oxazepam and found that in acid buffers it is reduced with the uptake of 4 electrons to form 7-chloro-5-phenyl-1,3,4,5-tetrahydro-2H-1,4-benzodiazepin-2-one at the dropping mercury electrode. However, in alkaline buffers the unstable 4,5 dihydro derivative which is formed first by consuming 2 electron, reacts further forming a cyclic aldehyde-ammonia adduct which undergoes reduction to form the same product as is formed in acidic solution.

## 6.8 Chromatographic Analysis

Many of the more common chromatographic techniques have been applied to oxazepam.

### 6.81 Paper Chromatography

Oxazepam has been chromatographed on Whatman #1 paper with either butanol, ethanol, water (17:3:20) upper phase or butanol, pyridine, water (6:4:3) as the eluant<sup>22</sup>.

### 6.82 Thin Layer Chromatography

The various eluant systems used for thin layer chromatography on silica gel plates for oxazepam are given in Table VII. Table VIII gives spray reagents used for the detection of oxazepam on thin layer chromatographs.

### 6.83 Gas Chromatography

Gas chromatography has been used to analyze oxazepam. The necessary data of the various methods are given as Table IX.

# OXAZEPAM

Table VII  
Thin Layer Chromatography System for Oxazepam

<u>R<sub>f</sub></u>	<u>Eluant</u>	<u>Reference</u>
0.00	chloroform:ethanol (29:1)	27
0.03	cyclohexane:diethylamine:benzene (75:20:15)	40
0.04	benzene:ethyl acetate (5:1)	41
0.08	benzene:ethanol:ammonium hydroxide (95:15:5)	40
0.08	chloroform:toluene:methanol (10:9:1)	42
0.13	heptane:chloroform:ethanol (10:10:1)	43
0.13	chloroform:cyclohexane:diethylamine (40:50:10)	44
0.14	toluene:nitromethane:methanol (11:8:1)	42
0.16	carbon tetrachloride:methanol (90:10)	44
0.21	benzene:methanol:ammonium hydroxide (90:10:1)	13
0.23	toluene:diethylamine (80:20)	44
0.29	benzene:acetone:diethylamine (70:20:10)	43
0.29	isopropanol:isopropyl ether (16:84)	45
0.35	chloroform:methanol (10:1)	42
0.37	ethanol:water (96:4)	45
0.46	methanol:acetone (12:88)	45
0.48	methanol:methyl acetate (18:82)	45
0.50	benzene:ethanol:diethylamine (5:1:0.5)	46
0.50	chloroform:ethanol:acetone (8:1:1)	41
0.51	cyclohexane:diethylamine (85:17)	44
0.52	carbon tetrachloride:methanol (75:25)	44
0.55	acetone	40
0.57	methanol:ammonium hydroxide (100:1.5)	40
0.58	chloroform:acetone:methanol (70:20:10)	44
0.59	heptane:chloroform:ethanol (5:5:2)	47
0.60	ethyl acetate:1,2 dichloroethane (80:20)	44
0.60	isopropanol:ammonium hydroxide (20:1)	43
0.66	benzene:acetone:methanol (55:35:10)	44
0.68	isopropanol:methanol (30:70)	44
0.68	chloroform:methanol:acetic acid (88:10:2)	44
0.73	isopropanol:ammonium hydroxide:water (75:17:18)	44
0.82	chloroform:acetone:diethylamine (50:40:10)	44
0.84	ethyl acetate:methanol:acetic acid (80:20:10)	44
0.88	chloroform:acetic acid:methanol (15:1:4)	41

Sadee and Van der Kleijn<sup>38</sup> found that oxazepam rearranged to the quinazoline carboxaldehyde (Figure 8, Structure IV) during gas chromatography.

#### 6.84 Column Chromatography

Scott and Bommer<sup>39</sup> describe a high pressure liquid chromatography system which was used for several benzodiazepines, including oxazepam. The column was 100 cm. x 1 mm. i.d. stainless steel packed with Waters Associates Durapak "OPN", 36-75  $\mu$ m. particle diameter. The eluant was a mixture of hexane and isopropanol. An ultra-violet detector was used.

Table VIII  
TLC Spray Reagents for the Detection of Oxazepam

<u>Reagent</u>	<u>Color</u>	<u>Light* Source</u>	<u>Reference</u>
Bromine water	orange	vis.	13
Reinecke solution	rose	"	13
Iodine solution	brown	"	13
Sulfuric acid	yellow	"	13
Dragendorff	yellow	"	46
Chlorine-o-toluidine	violet	"	42
Cerric sulfate-Dragendorff	red-orange	"	42
Diphenylcarbazone	light purple	"	48
Silver acetate	blue-purple	"	48
Mercuric sulfate	lavender	"	48
Cinnamaldehyde	blue	UV	40
Furfural reagent	green	"	40
Zinc (II) chloride-hydrochloric acid	beige	"	44
70% perchloric acid	yellow-orange	vis.	44
Cerium (IV) sulfate	yellow	UV	44
Formaldehyde-hydrochloric acid	light blue	"	44
40% o-phosphoric acid	light yellow	vis.	44
Antimony (III) chloride-acetic acid	light blue	UV	44
	yellow	vis.	44
Vanillin-sulfuric acid	blue	UV	44
	yellow	vis.	44

\*vis. = visible

UV = ultraviolet



Table IX

Gas Chromatographic System for Oxazepam

<u>Column Packing</u>	<u>Column</u>	<u>Carrier Gas</u>	<u>Column Temperature</u>	<u>Detector</u>	<u>Ref.</u>
3% OV 1 on Gas Chrom Q (60/80)	2 m. x 2 mm. glass	Nitrogen @ 22 ml./min.	245°C.	flame ionization	49
3% OV 17 on Gas Chrom Q (60/80)	4 ft. x 4 mm. glass	Argon-methane (90:10) @ 100 ml/min.	230°C.	electron capture	50
1.5% OV 1 on HP Chrom G	3 ft. x 1/8 in. glass	Helium @ 20 ml./min.	200 - 280°C. programmed	total ion current	38
3% OV 17 on Gas Chrom W	6 ft. x 1/8 in. stainless steel	Nitrogen @ 70 ml./min.	245°C.	flame ionization	38
3% OV 17 on Gas Chrom W	6 ft. x 1/8 in. stainless steel	Argon-methane (20:1) @ 70 ml./min.	245°C.	electron capture	38

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## **PHENAZOPYRIDINE HYDROCHLORIDE**

*Kenneth W. Blesel, Bruce C. Rudy, and Bernard Z. Senkowski*

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### Analytical Profile - Phenazopyridine Hydrochloride

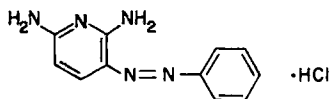
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# PHENAZOPYRIDINE HYDROCHLORIDE

## 1. Description

### 1.1 Name, Formula, Molecular Weight

Phenazopyridine hydrochloride is 2,6-diamino-3-(phenylazo)pyridine monohydrochloride.



$C_{11}H_{11}N_5 \cdot HCl$

Molecular Weight: 249.70

### 1.2 Appearance, Color, Odor

Phenazopyridine hydrochloride is a light to dark red, odorless crystalline powder.

## 2. Physical Properties

### 2.1 Infrared Spectrum

The infrared spectrum of a sample of reference standard phenazopyridine hydrochloride is shown in Figure 1 (1). The instrument used was a Perkin Elmer Model 621 recording spectrophotometer. The sample was dispersed in mineral oil in order to record the spectrum. The following assignments have been made for the bands in Figure 1 (1).

<u>Band</u>	<u>Assignment</u>
3329 and 3275 $\pm$ 5 $cm^{-1}$	N-H stretch
3065 $\pm$ 5 $cm^{-1}$	aromatic C-H stretch
1601 and 1500 $\pm$ 5 $cm^{-1}$	aromatic ring vibrations
1638 $\pm$ 5 $cm^{-1}$	NH <sub>2</sub> deformations
815 $\pm$ 5 $cm^{-1}$	out of plane deformation of H on pyridine ring
713 $\pm$ 5 $cm^{-1}$	out of plane bending of NH <sub>2</sub>
682 $\pm$ 5 $cm^{-1}$	out of plane deformations of H on benzene ring

### 2.2 Nuclear Magnetic Resonance Spectrum (NMR)

The NMR spectrum of phenazopyridine hydrochloride is shown in Figure 2 (2). The solvent used was DMSO-d<sub>6</sub>, the internal reference was tetramethylsilane and the

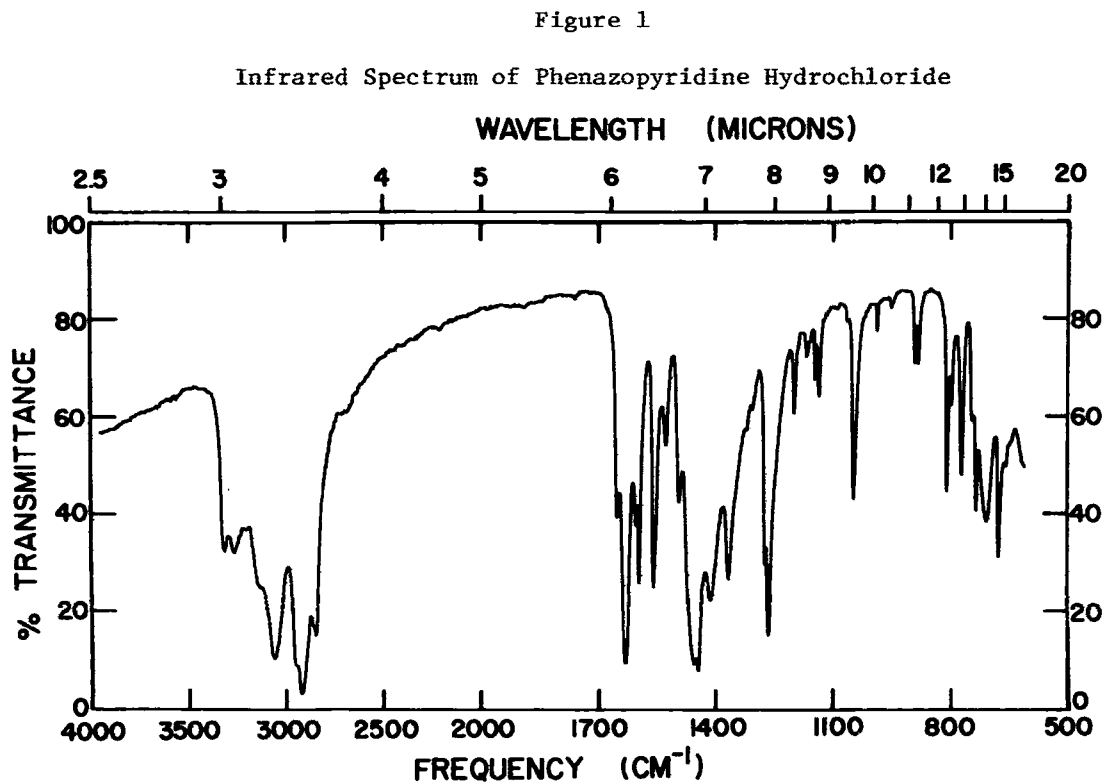
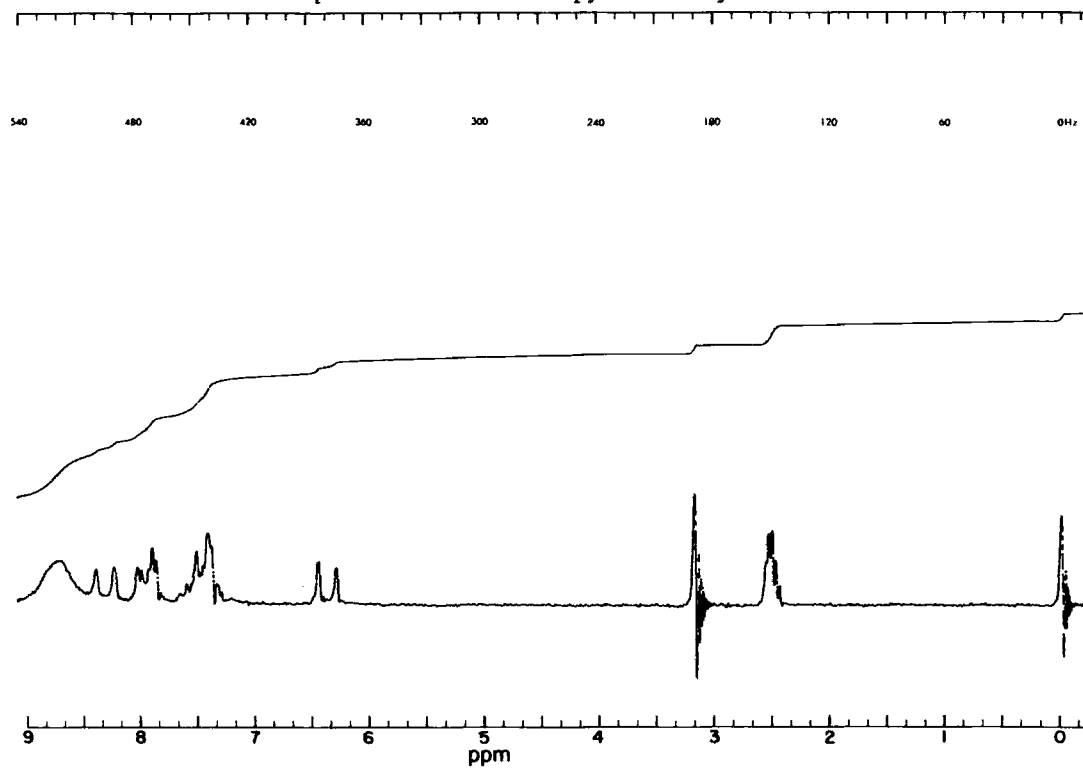




Figure 2

NMR Spectrum of Phenazopyridine Hydrochloride



solution concentration was 30.9 mg in 0.5 ml of solvent. The spectral assignments are given in Table I (2).

Table I

## NMR Spectral Data for Phenazopyridine Hydrochloride

<u>Proton</u>	<u>No. of Each</u>	<u>Chemical Shift (ppm)</u>	<u>Multiplicity</u>	<u>Coupling Constant</u>
Proton on C <sub>5</sub>	1	6.36	doublet	$J_{H_5-H_4}=10$ Hz
Protons meta and para to phenylazo nitrogen	3	7.25-7.65	multiplet	
Protons ortho to phenylazo nitrogen	2	7.85-8.02	multiplet	
Proton on C <sub>4</sub>	1	8.29	doublet	
Amino protons	4	$\sim 8.7$	singlet (broad)	

2.3 Ultraviolet Spectrum

The ultraviolet spectrum of phenazopyridine hydrochloride (0.5 mg/100 ml of acidified 3A alcohol) in the region of 210-450 nm exhibits two maxima and three minima. The maxima occur at 238-240 nm ( $\epsilon = 2.2 \times 10^4$ ) and 390-392 nm ( $\epsilon = 2.4 \times 10^4$ ), while the minima are at 220 nm, 272 nm and 296 nm respectively (3). The spectrum is shown in Figure 3.

2.4 Fluorescence Spectrum

The excitation and emission spectra of phenazopyridine hydrochloride (10  $\mu$ g/ml in methanol) are shown in Figure 4 (4). The instrument used was a Farrand MK-1 recording spectrofluorometer. An excitation wavelength of 341 nm produced an emission spectrum with a maximum at 380 nm.

2.5 Mass Spectrum

The low resolution mass spectrum of phenazopyridine is shown in Figure 5 (5). The spectrum was obtained using a CEC 21-110 spectrometer with an ionizing voltage of 70 eV, which was interfaced with a Varian data

Figure 3

Ultraviolet Spectrum of Phenazopyridine Hydrochloride

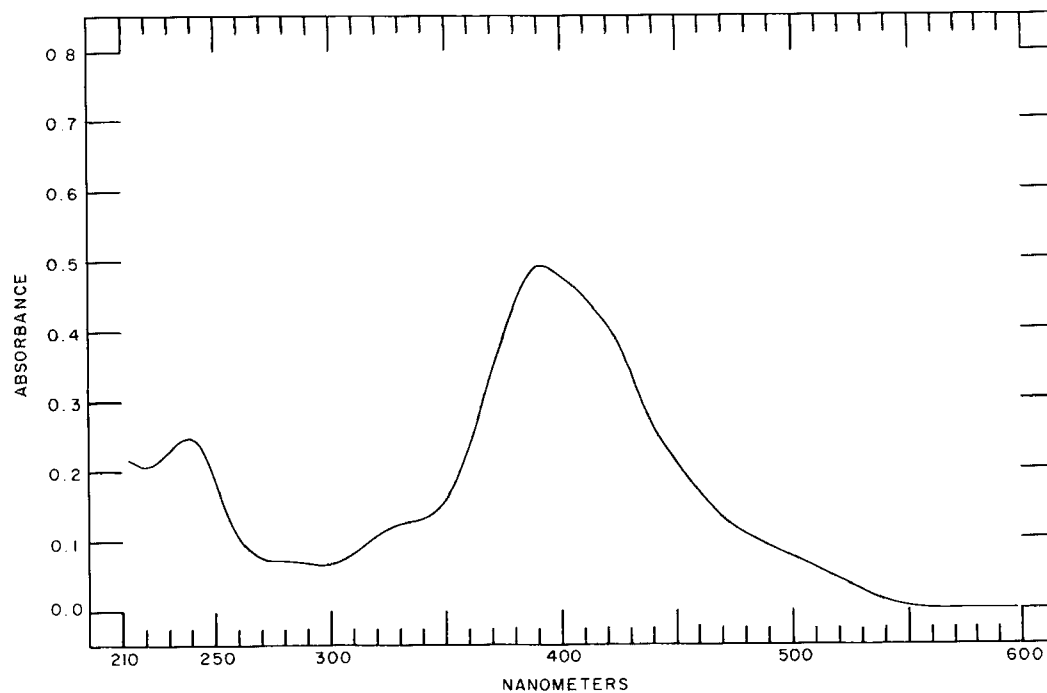


Figure 4

Fluorescence Spectra of Phenazopyridine Hydrochloride

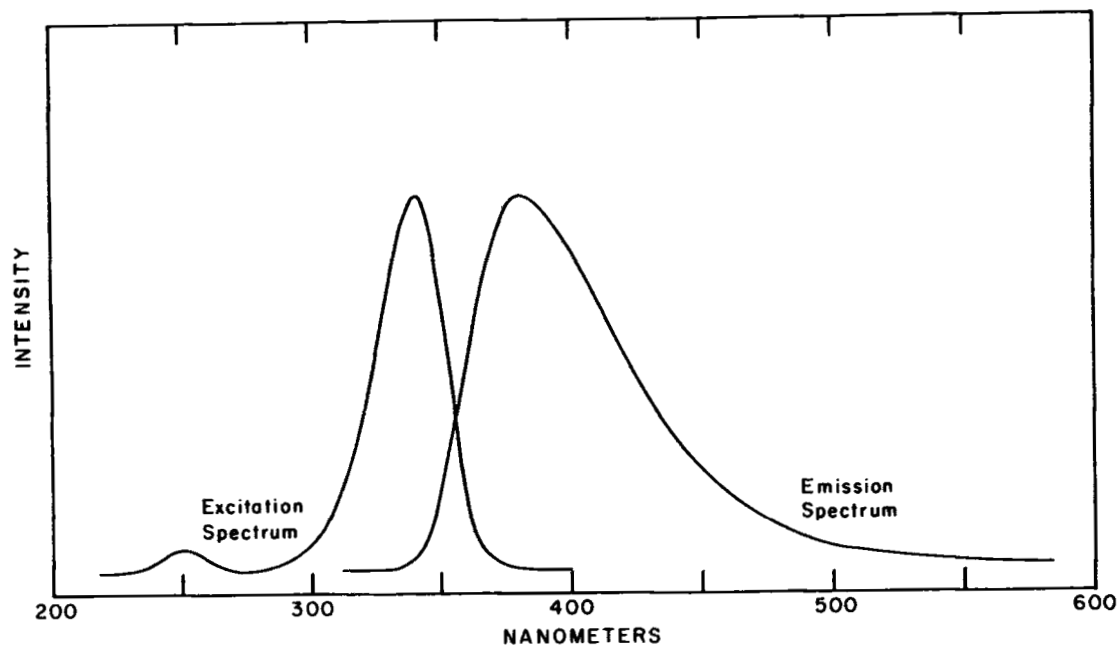
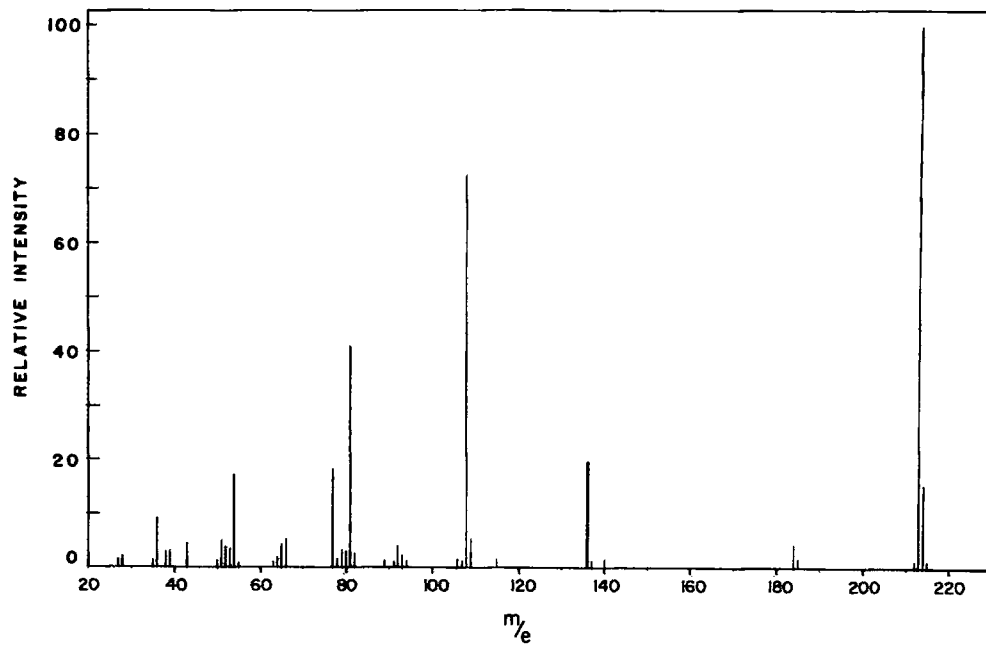


Figure 5

Mass Spectrum of Phenazopyridine



system 100 MS. The data system accepted the output of the spectrometer, calculated the masses, compared the intensities to the base peak and plotted this information as a series of lines whose heights were proportional to the intensities.

The molecular ion of the free base was measured at  $m/e$  213. Other characteristic masses were observed at  $m/e$  214, corresponding to  $(M+H)$ ,  $m/e$  184, which corresponds to the loss of  $HN_2$  from the molecular ion,  $m/e$  136, the loss of a phenyl ring from the parent mass, and  $m/e$  108 which is the 2,6-diamino-pyridinium moiety (5). A high resolution scan confirmed the results of the low resolution spectrum.

#### 2.6 Optical Rotation

Phenazopyridine hydrochloride exhibits no optical activity.

#### 2.7 Melting Range

Phenazopyridine hydrochloride melts with decomposition at approximately  $235^{\circ}\text{C}$  when a class Ia procedure is used (6).

#### 2.8 Differential Scanning Calorimetry (DSC)

The DSC scan of phenazopyridine hydrochloride is shown in Figure 6 (7). The curve was obtained with a Perkin Elmer DSC-1B Calorimeter. The temperature program used was  $10^{\circ}\text{C}/\text{min.}$  in an atmosphere of nitrogen. Under these conditions, the exothermic decomposition of phenazopyridine hydrochloride occurs at approximately  $239^{\circ}\text{C}$ .

#### 2.9 Thermogravimetric Analysis (TGA)

The TGA scan showed no loss of weight as the temperature was raised from ambient to  $115^{\circ}\text{C}$  at a rate of  $10^{\circ}\text{C}/\text{min.}$  (7).

#### 2.10 Solubility

The solubility data obtained for a sample of reference standard phenazopyridine hydrochloride at  $25^{\circ}\text{C}$  is shown in Table II (8). The equilibration time was 20 hours at  $25^{\circ}\text{C}$ .

Figure 6

DSC Curve for Phenazopyridine Hydrochloride

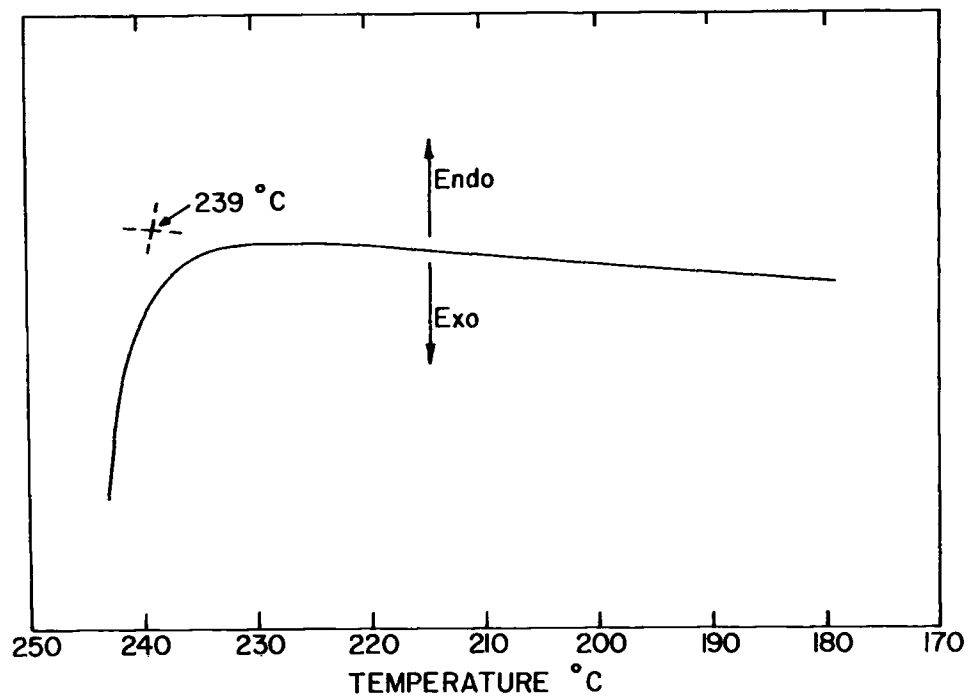


Table II

## Phenazopyridine Hydrochloride - Solubilities

<u>Solvent</u>	<u>Solubility (mg/ml)</u>
3A alcohol	2.7
benzene	0.3
chloroform	0.4
95% ethanol	3.5
diethyl ether	0.2
2-propanol	2.1
methanol	2.7
petroleum ether (30°-60°)	0.1
water	3.2
1N HCl	0.3
acidified 3A alcohol	3.2

2.11 Crystal Properties

The x-ray powder diffraction data from phenazopyridine hydrochloride is presented in Table III (9). The operating conditions of the instrument are given below.

Instrument Conditions

## General Electric Model XRD-6 Spectrogoniometer

Generator:	50 KV, 12-1/2 MA
Tube target:	Copper
Radiation:	Cu $K_{\alpha}$ = 1.542 Å
Optics:	0.1° Detector slit
	M.R. Soller slit
	3° Beam slit
	0.0007" Ni filter
	4° take off angle
Goniometer:	Scan at 0.2° 2θ per minute
Detector:	Amplifier gain - 16 coarse,
	8.7 fine
	Sealed proportional counter
	tube and DC voltage at
	plateau
	Pulse height selection $E_L$ -
	5 volts;
	$E_u$ - out



# PHENAZOPYRIDINE HYDROCHLORIDE

Rate meter T.C. 4  
 2000 C/S full scale  
 Recorder: Chart Speed 1 inch per 5 minutes  
 Samples: Prepared by grinding at room temperature

Table III

Interplanar Spacings in Phenazopyridine Hydrochloride  
 from X-ray Powder Diffraction Data

<u>2θ</u>	<u>d*</u>	<u>I/I<sub>0</sub>**</u>	<u>2θ</u>	<u>d*</u>	<u>I/I<sub>0</sub>**</u>
8.68	10.20	6	27.88	3.20	85
9.12	9.70	30	29.28	3.05	18
10.22	8.66	84	30.64	2.92	23
12.88	6.87	10	31.32	2.86	18
13.68	6.47	8	32.84	2.73	10
14.46	6.13	100	34.38	2.61	6
18.10	4.90	20	36.06	2.49	9
19.44	4.57	32	36.58	2.46	5
20.40	4.35	19	37.58	2.39	6
21.82	4.07	25	40.02	2.25	4
22.10	4.02	21	41.14	2.19	3
23.06	3.86	10	42.32	2.14	8
24.06	3.70	10	43.78	2.07	2
25.74	3.46	32	47.00	1.93	4
26.38	3.38	85	48.75	1.87	3
27.10	3.29	46			

$$*d = (\text{interplanar spacing}) \frac{n\lambda}{2 \sin \theta}$$

\*\*I/I<sub>0</sub> = relative intensity (based on highest intensity of 100)

## 3. Synthesis

Phenazopyridine may be prepared by coupling benzene diazonium chloride with α,α-diamino pyridine (10).

## 4. Stability Degradation

Phenazopyridine has been found to be stable in distilled water and 0.1N sodium hydroxide when refluxed for one hour on a steam bath. Some degradation occurs in 0.1N

hydrochloric acid solution at reflux temperature. After one hour at reflux it was found, by quantitative densitometry on a thin-layer chromatographic plate, that 20-25% of the initial amount of material had degraded. The degradation products were yellow but no identification was attempted.

## 5. Drug Metabolic Products

The metabolic fate of phenazopyridine hydrochloride has been studied in the rabbit and in man (11). When an oral dose of 600 mg was given to humans, about 80% was eliminated in the urine within 24 hours. Of this amount, 7.6% appeared as aniline, 19.9% as N-acetyl-p-aminophenol, 27.1% as p-aminophenol, 45.4% as the unchanged drug as well as a trace amount of o-aminophenol. Triaminopyridine also was detected but not measured.

## 6. Methods of Analysis

### 6.1 Elemental Analysis

The results of an elemental analysis of a sample of reference standard phenazopyridine hydrochloride are presented in Table IV (12).

Table IV

Elemental Analysis of Phenazopyridine Hydrochloride

<u>Element</u>	<u>Theoretical %</u>	<u>Found %</u>
C	52.91	52.89
H	4.84	4.76
N	28.05	28.00
Cl	14.20	14.23

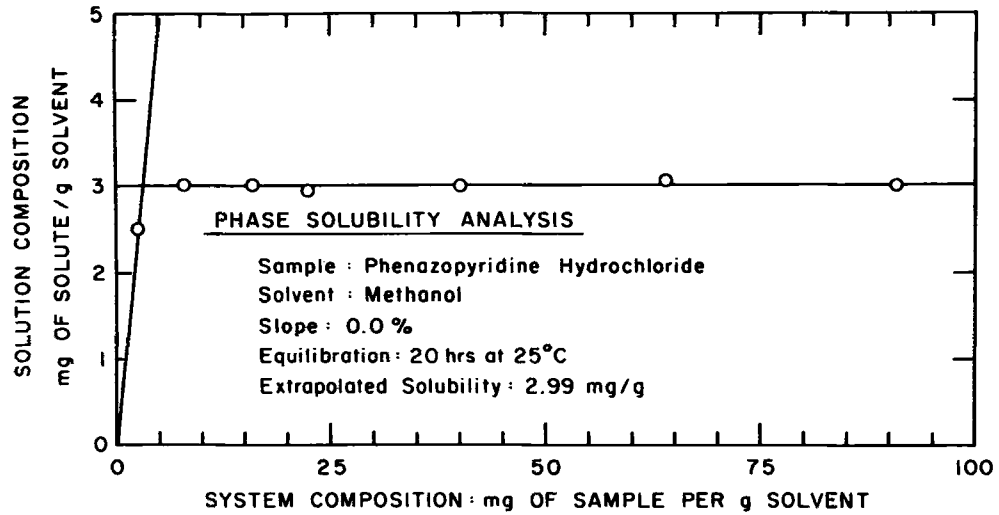
### 6.2 Phase Solubility Analysis

Phase solubility analyses have been carried out for phenazopyridine hydrochloride. An example is shown in Figure 7 (8). The solvent used was methanol with an equilibration time of 20 hours at 25°C.

### 6.3 Thin Layer Chromatographic Analysis

Phenazopyridine can be detected in AZO GANTRISIN tablets using the following TLC procedure. The tablets

Figure 7



are ground to a powder and a portion dissolved in acetone. This is applied to the plate (silica gel GF) and developed for at least 10 cm with the solvent mixture chloroform: heptane:3A alcohol (45:45:10) in a paper-lined, pre-saturated tank. Phenazopyridine can be detected visually as a yellow spot at an  $R_f$  of 0.4 (13).

#### 6.4 Direct Spectrophotometric Analysis

The spectrophotometric determination of phenazopyridine hydrochloride in 1.0N HCl is, according to the National Formulary, the method of choice for the assay of the bulk drug (14). The sample is dissolved in 1.0N HCl and the absorbance determined at the maximum at about 480 nm. The quantity of phenazopyridine hydrochloride is calculated by comparison with a sample of reference standard material prepared and measured in a similar way.

The above method is subject to the disadvantage of the low solubility of phenazopyridine hydrochloride in 1.0N HCl. The substitution of acidified 3A alcohol as the solvent would give approximately a ten-fold increase in the solubility and eliminate the necessity of heating the solution in order to dissolve the required amount of phenazopyridine hydrochloride. The maximum in this solvent occurs about 390 nm.

#### 6.5 Coulometric Analysis

Phenazopyridine hydrochloride may be determined coulometrically in an acidified water-acetone solution, utilizing a mercury pool electrode. The sample is reduced at a potential of -0.40 volts until the observed cell current drops to 1/1000 of its initial value. A blank determination is carried out and any corrections made. Each coulomb of electricity is equivalent to 646.9 mcg of phenazopyridine hydrochloride (6).

#### 6.6 Titrimetric Analysis

Phenazopyridine hydrochloride may be assayed by a potentiometric titration with  $\text{HClO}_4$ . The sample is dissolved in water which is made basic with 10% NaOH and the liberated base is extracted into chloroform. It is then titrated potentiometrically with 0.01N  $\text{HClO}_4$ , in dioxane, using a glass-calomel (sleeve type) electrode combination. Each ml of 0.01N  $\text{HClO}_4$  is equivalent to 2.497 mg of phenazopyridine hydrochloride (15).

## PHENAZOPYRIDINE HYDROCHLORIDE

### 7. Acknowledgments

The authors wish to acknowledge the Scientific Literature Department and the Research Records Office of Hoffmann-La Roche Inc. for assistance in the literature search for this Analytical Profile.

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**PHENYLEPHRINE HYDROCHLORIDE**

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Reviewed by E. L. Pratt and L. Chafetz

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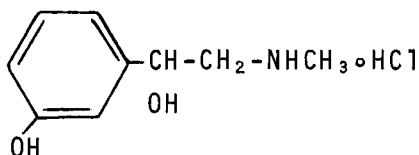
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## 1. Description

### 1.1 Name, Formula, Molecular Weight

Phenylephrine Hydrochloride is 1-*m*-Hydroxy- $\alpha$ -[(methylamino)methyl] benzyl alcohol hydrochloride (1). It is also known as 1- $\alpha$ -hydroxy- $\beta$ -methylamino-3-hydroxy-1-ethylbenzene hydrochloride; *m*-methylaminoethanolphenol hydrochloride; Neo Synephrine hydrochloride; Meta-Synephrine hydrochloride; Adrianol; *m*-Sympatol; Meta-Sympatol; Neophryn; Isophrin Hydrochloride; Oftalfrine; Lexatol (2).



$C_9H_{11}ClNO_2$

Mol. Wt. 203.67

### 1.2 Appearance, Color, Odor, Taste

White or nearly white, odorless crystals having a bitter taste.

## 2. Physical Properties

### 2.01 Melting Range

Phenylephrine HCl	140 - 145°C (1)*
	139 - 143°C (3)
Phenylephrine base	170 - 177°C (1)*
	170 - 171°C (3)

\*USP Specifications

### 2.02 Solubility

(1). Freely soluble in water and in alcohol

### 2.03 pK

$pK_1 = 8.77$  (4)

$pK_2 = 9.84$

### 2.04 Optical Rotation

$[\alpha]_D^{25} = -46.2$  to  $-47.2^\circ$  ( $c=1$ ) (2)

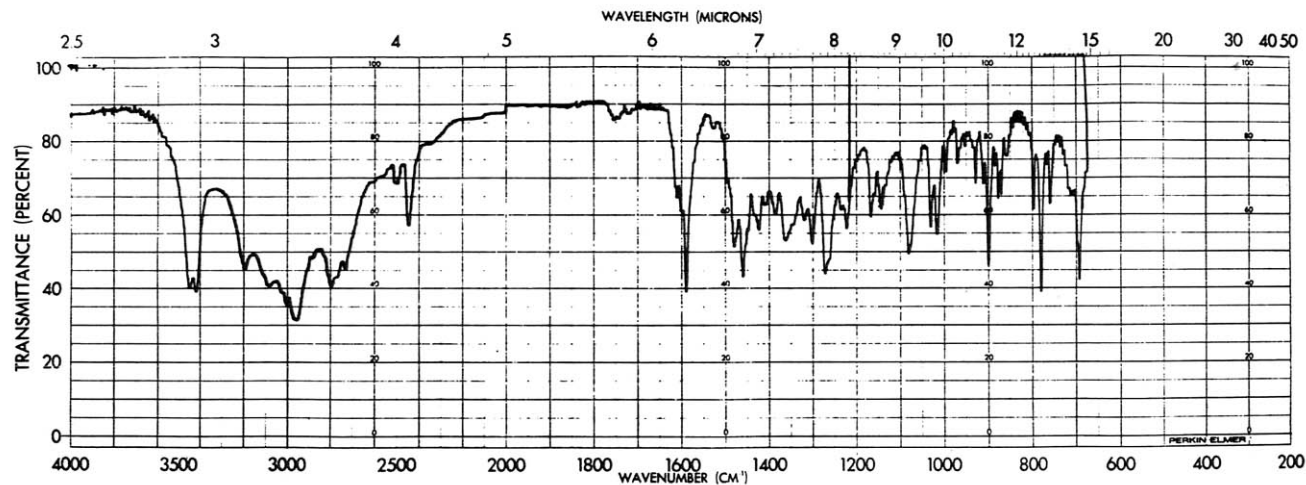


Fig. 1 Phenylephrine hydrochloride - IR spectrum of 13 mm. KBr pellet from 1 mg. drug dispersed in 200 mg. KBr - Instrument: Perkin-Elmer 621

# PHENYLEPHRINE HYDROCHLORIDE

## 2.05 Ultraviolet Spectrum

<u>Solution</u>	<u><math>\lambda</math> max. nm.</u>	<u><math>\epsilon \times 10^{-3}</math></u>
0.05 <i>N</i> HCl	216	5.91
	274	1.81
	279s	1.65
0.05 <i>N</i> NaOH	239	8.95
	292.5	3.04
Isosbestic Points	222.5	4.28
	260	6.66
	278.5	1.67

## 2.06 Infrared Spectrum (Figure 1)

The spectrum in Figure 1 was obtained using a Perkin-Elmer 621, Infrared Spectrophotometer. A 13 mm. KBr pellet containing 1 mg. phenylephrine HCl and 200 mg. KBr was used. Characteristic band assignments are listed below.

<u>Band <math>\text{cm}^{-1}</math></u>	<u>Assignment</u>
3420, 3450	-OH
2400-2800	NH $\frac{1}{2}$
1590	aromatic
1270	C-O stretch aromatic
1080	C-O stretch secondary alcohol
900	aromatic out of plane bend single CH
780	aromatic out of plane bend meta disubstituted
690	aromatic out of plane bend meta disubstituted

## 2.07 Nuclear Magnetic Resonance Spectrum

Solvent: DMSO

Instrument: Varian A60

Concentration: Approximately 8%

Phenylephrine Hydrochloride (Figure 2)

<u>ppm. (from TMS)</u>	<u>Integral</u>	<u>Identification</u>
2.6 (S)	3	N-CH <sub>3</sub>
2.8 - 3.2 (M)	2	CH <sub>2</sub> -N
4.9 (M)	1	CH
6.1 (B)*	1	CHOH
6.7 - 7.5 (M)	4	aromatic
9 - 9.8 (B)*	3	phenolic OH, NH <sub>2</sub> <sup>+</sup>

Phenylephrine Base (Figure 3)

<u>ppm. (from TMS)</u>	<u>Integral</u>	<u>Identification</u>
2.4 (S)	3	N-CH <sub>3</sub>
2.55 (D)	2	CH <sub>2</sub> -N
4.5 (T)	1	CH
4.8 - 5.8 (B)*	3	OH (2), NH
6.5 - 7.5 (M)	4	aromatic

\*Disappear on D<sub>2</sub>O exchange

2.08 Mass Spectrum

The low resolution mass spectrum was determined on an MS 902 mass spectrometer. The sample was introduced by a heated direct insertion probe at a temperature of 150°C for the hydrochloride and of 100°C for the base.

<u>Mass No.</u>	<u>Intensity HCl</u>	<u>Intensity Base</u>
167	10	38
148	2	6
133	1	4
121	5	10
107	2	10
95	4	20
77	8	15
65	4	30
44	100	100
38	8	---
36	26	---

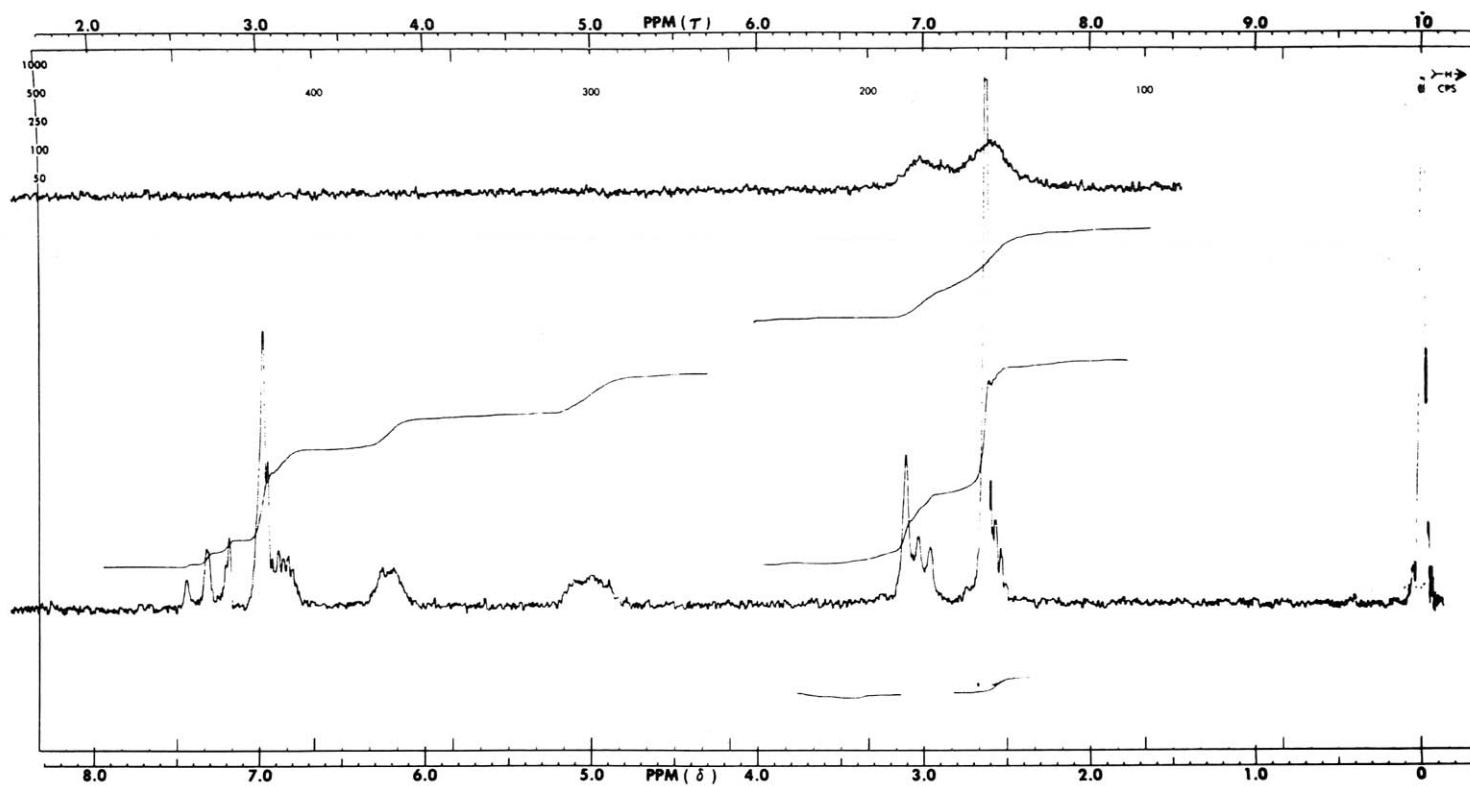


Fig. 2 Time averaged NMR spectrum of phenylephrine hydrochloride, 8% in DMSO - Instrument: Varian A-60

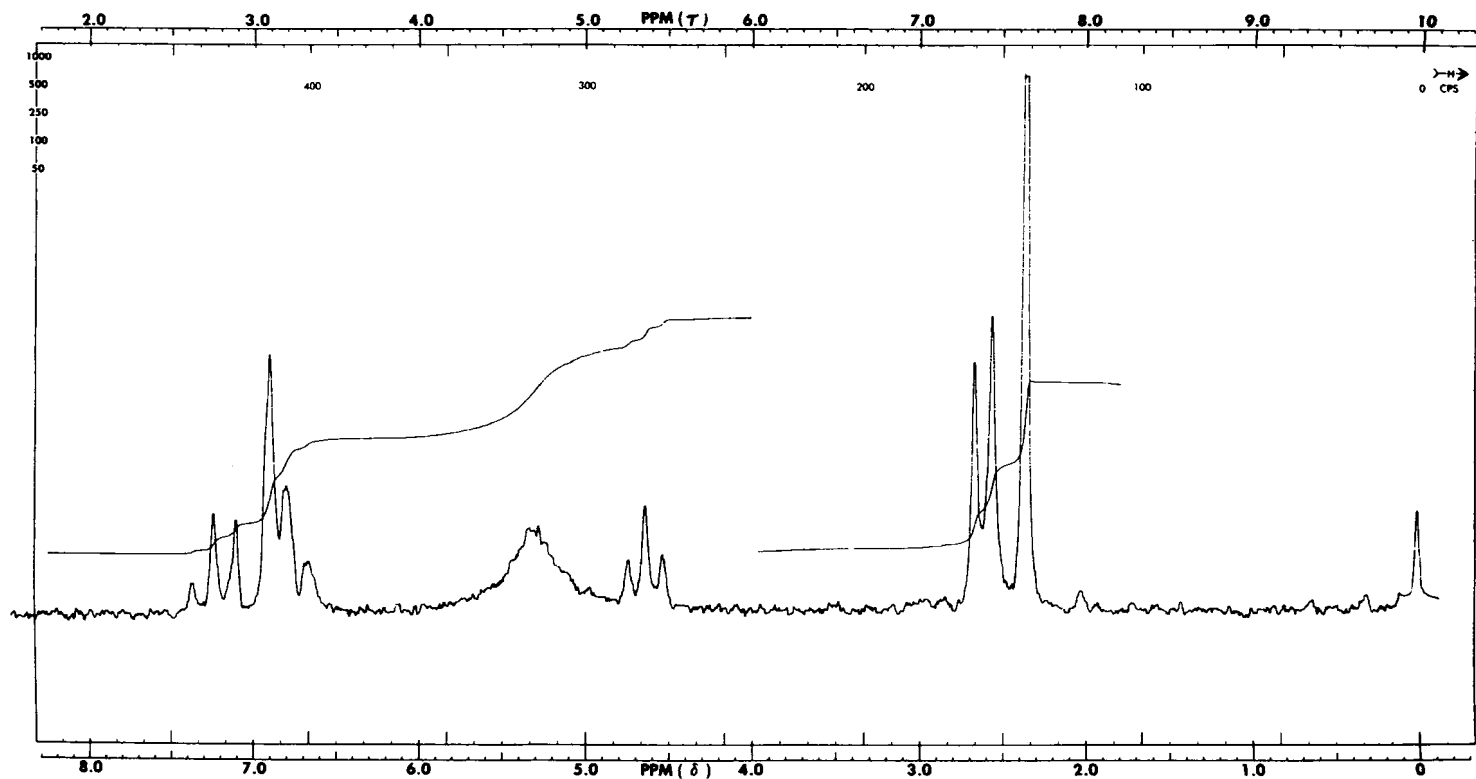


Fig. 3 Time averaged NMR spectrum of phenylephrine base, about 8% in DMSO - Instrument: Varian A-60

2.09 Differential Thermal Analysis

	<u>Onset of Melting Peak Endotherm</u>	
Phenylephrine HCl	142°C	144°C
Phenylephrine Base	172°C	174°C

2.10 Thermal Gravimetric Analysis

No significant weight loss until decomposition at 230°C for phenylephrine HCl.

3. Synthesis

Legerlotz was the first to prepare phenylephrine hydrochloride by the hydrogenation of *m*-hydroxy-*o*-methylamino-acetophenone in the presence of colloidal palladium (5). Bergmann and Sulzbacher (6) synthesized phenylephrine by treating 5-(3'-benzyloxyphenyl)-3-methyl-2-oxazolidone with 40% hydrochloric acid solution. Russell and Childress (7) achieved the same end by refluxing 3-benzyloxy-*N*-methylmandelamide with LiAlH<sub>4</sub> in tetrahydrofuran (THF) to produce 3-benzyloxy- $\alpha$ -methylamino-methylbenzyl alcohol hydrochloride. This compound was then hydrogenated in the presence of 5% palladium-C catalyst until one equivalent of H is consumed. The hydrogenation of 3-benzyloxy- $\alpha$ -methylamino-methylbenzyl alcohol was also used by Britten (8) and, most recently, Rizzi (9) as the last steps of their syntheses of phenylephrine.

4. Stability - Degradation

Phenylephrine hydrochloride is stable as a solid. The degradation of aqueous solutions has been studied by El-Shibini *et al.* (10,11). The compound is stable below pH 7. Above pH 7, degradation occurs and apparently involves the side chain with loss of the secondary amine function. The phenolic group remains intact. The decomposition products have not been identified but 5-hydroxy-*N*-methylindoxyl has been proposed. The presence of heavy metals, particularly copper was found to catalyze the decomposition.

Troup and Mitchner (12) characterized the acetylation of phenylephrine in the presence of aspirin. The reaction is apparently accelerated by the presence of phenylephrine base and the availability of acetate. The amine group acetylates preferentially. The hydroxyl groups become acetylated after prolonged exposure to aspirin.

Luduena *et al.* (13) studied the effect of ultraviolet irradiation on phenylephrine solutions. Epinephrine was identified as the product. Luduena postulated the epinephrine could further react to produce other compounds. The findings of West and Whittet (14) support this postulate.

Schriftman (15) found from 12 to 28% decomposition of unbuffered phenylephrine solutions in one week at various temperatures. He also found up to five decomposition products. The secondary amine function was absent in at least one of the products.

Broadly and Roberts (16) found a second compound present in strong acid (10 *N* hydrochloric acid) solutions of phenylephrine. The compound was not identified.

Misgen (17) determined the physical compatibility of phenylephrine with twenty-seven common intravenous admixtures. He found on adding a solution of phenylephrine to a solution of dilantin sodium a precipitate formed within two hours.

Fagard (18) found phenylephrine solutions to be stable in brown glass bottles, give 1% decomposition after 11 days in low density polyethylene bottles and decompose to 81% of initial when stored for 130 days in nylon bottles.

Petraglia and Dick (19) reported the stabilization of phenylephrine solutions to sunlight by adding 0.2% sodium metabisulfite and 0.1% tartrazine.

El-Shibini *et al.* (11) indicated the stabi-



lizing effect of EDTA on basic solutions of phenylephrine.

Kisbye and Bols (20) found that no racemization of phenylephrine occurs as a function of pH. Pratt (21) found phenylephrine optically stable in solutions at pH 3.0 and 6.0 when refluxed for 3 hours.

Fourneau *et al.* (22) reported the reaction of phenylephrine with aldehydes under "physiological conditions" to produce a mixture of 4,6- and 4,8-dihydroxy-2-methyl-1,2,3,4-tetrahydroisoquinolines.

## 5. Drug Metabolic Products

Relatively little work has been reported on the metabolism of phenylephrine. Bruce (23) reported phenylephrine is excreted in urine almost entirely as the sulfate conjugate. Bruce reported 82% average total recovery of phenylephrine from a tablet formulation in 24 hours. Typical urine sample contained from 86 to 98% of the recovered phenylephrine as the sulfate conjugate.

Bogner and Walsh (24), and Cavallito *et al.* (25) showed blood level and urine excretion patterns for phenylephrine hydrochloride and phenylephrine tannate. Their work involved tritium labeled drug. Metabolic products were not identified.

Rubin and Knott (26) reported a fluorometric procedure for determining phenylephrine in serum.

Dombrowski and Pratt (27) report a procedure for determining unmetabolized phenylephrine in plasma.

## 6. Methods of Analysis

### 6.1 Direct Spectrophotometric Analysis

The ultraviolet absorption band at 272

nm. is due to the phenolic structure. The absorbance can be used to quantitate phenylephrine directly (28,29,30) or after extraction (31). Shifting the maxima to 290 nm. in basic solution has also been used as a direct assay as well as a differential technique to quantitate phenylephrine (32,33). Oxidation of phenylephrine to *m*-hydroxy benzaldehyde and measuring absorbance at 257 nm. and/or 315 nm. in acidic or neutral solutions or at 237, 267 and 357 nm. in basic solution has also been carried out (34). The oxidation also offers increased sensitivity over direct U.V. U.V. is a common detection technique for thin layer, paper and column chromatographic techniques.

## 6.2 Colorimetric Analysis

Phenylephrine has been identified and quantitated by a variety of colorimetric techniques.

### 6.21 Indophenol Dye

Indophenol dye is formed by the reaction of  $p\text{-Me}_2\text{NC}_6\text{H}_4\text{NH}_2\text{Cl}$  and  $\text{K}_3\text{Fe}(\text{CN})_6$  with para unsubstituted phenols in alkaline media (35, 36,37). The dye resulting from the reaction with phenylephrine has an absorbtion maximum about 620 nm.

### 6.22 Coupling with *p*-Nitroaniline

Phenylephrine may be coupled with diazotized *p*-nitroaniline in acid solution (38, 39). The resulting compound is made basic and determined at 495 nm.

### 6.23 Coupling with 4-Aminoantipyrine

4-aminoantipyrine is a selective coupling agent for phenols with the para position free. The reaction is carried out in alkaline buffer solution pH  $\approx$  9 in the presence of  $\text{K}_3\text{Fe}(\text{CN})_6$ . The resulting absorbtion maximum at 460 nm. is quantitative for phenylephrine (40,41,42).

### 6.24 Complexation

Phenylephrine forms complexes with

various sulfophthalein dyes in neutral to slightly basic solution. The resulting complexes are then extracted into a polar organic solvent and the color determined spectrophotometrically (43, 44).

#### 6.25 Coupling with Nitrous Acid

When solutions of phenylephrine are heated with mercury salts then coupled with nitrous acid, a red color develops. The peak at 495 nm. has been used to quantitate phenylephrine (45,46). Detailed studies of the reaction conditions have been conducted (47,48).

#### 6.26 Identification

Phenylephrine undergoes many color reactions. Several schemes for identifying phenylephrine alone and in the presence of other drugs have been developed (49,50,51,3).

#### 6.27 Other Methods

Many other quantitative color reactions have been reported in the literature. The reaction product with iodic acid is determined at 420 nm. (50). Phenylephrine reacts with 2,6-dichloroquinone in neutral solution and is determined at 625 nm. (52). The oxidation of phenylephrine to an aldehyde followed by reaction with thiobarbituric acid (53) or 3-methylbenzothiazolin-2-one (54) is also quantitative. Ninhydrin reacts with phenylephrine to produce a pink color with a maximum absorbance at 440 nm. (55).

### 6.3 Chromatographic Methods of Analysis

#### 6.31 Paper Chromatography

Paper chromatography has been used to isolate phenylephrine from its decomposition products (15,56) and from other sympathicomimetics (57,58,59,60,37). Table 6.31-1 summarizes the literature for paper chromatographic separation of phenylephrine.

TABLE 6.31-1

<u>Solvent System</u>	<u>Method of Visualization</u>	<u>Rf x 100</u>	<u>Reference</u>
A	ninhydrin	57	57
B	diazotized <i>p</i> -sulfanilic dragendorff U.V. denitometry	63	15
C	ninhydrin	67	56
D	not available	--	60
E	indophenol	10	37

A = *n*-butanol/acetic acid/water 4:5:1

B = *n*-butanol/acetic acid/water 5:1:3

C = phenol containing 15% v/v 0.1 *N* HCl

D = butanol/toluene/acetic acid/water  
100:100:50:50

E = benzyl alcohol/acetic acid/water 5:2:5

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TABLE 6.32-1

<u>System</u>	<u>Rf x 100</u>	<u>Method of Detection</u>	<u>Reference</u>
I	5	spray 1% I <sub>2</sub> in methanol and/or dragendorff's reagent	61
II	21	"	61
III	33	"	61
IV	60	"	61
V	45	"	61
VI	--	potassium ferricyanide 0.6% w/v in 0.5% w/v NaOH quan. UV. densitometry	62
VII	50	ninhydrin	63

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	<u>Silica Gel Plates Coated with</u>		<u>Developing Solvent</u>
I	0.1	NaOH	cyclohexane/benzene/diethylamine 75:15:10 (v/v)
II	0.1 M	NaOH	methanol
III	0.1 M	NaOH	acetone
IV	0.1 M	KHSO <sub>4</sub>	methanol
V	0.1 M	KHSO <sub>4</sub>	95% ethanol

TABLE 6.32-1 (continued)

	<u>Silica Gel Plates</u> <u>Coated with</u>	<u>Developing Solvent</u>
VI	cellulose 250 $\mu$	<i>n</i> -butanol/acetic acid/water 4:1:5 v/v organic phase as the developing sol- vent
VII	silica gel G	<i>n</i> -butanol/acetic acid/water 12:1:7 v/v organic phase as the developing sol- vent

### 6.32 Thin Layer Chromatography

The thin layer chromatographic  $R_f$  values for phenylephrine in a number of solvent systems are given in Table 6.32-1

### 6.33 Liquid-Liquid Chromatography

Phenylephrine lends itself readily to liquid-liquid chromatography. The difficulty in extracting phenylephrine from aqueous solutions has been used to advantage to remove other compounds from phenylephrine. Levine and Doyle (64,65) and Cox (66) presented the theoretical aspects of liquid-liquid partition systems. Their work includes the partition coefficients of many drugs and is presented so that optimum conditions for particular separations may be selected. Table 6.33-1 summarizes the practical applications of liquid-liquid chromatographic separations of phenylephrine.

### 6.34 Gas Chromatography

Gas chromatography has been used to separate, identify and quantitate phenylephrine. A summary of the gas chromatographic data is presented in Table 6.34-1.

### 6.35 Ion Exchange Chromatography

Table 6.35-1 summarizes the literature on ion exchange separation of phenylephrine.

## 6.4 Spectrofluorometric and Phosphorimetric Analysis

Phenylephrine has native fluorescent properties. Udenfriend *et al.* (80) reported 270 nm. as the wavelength of excitation with 305 nm. being the wavelength of emission. The fluorescence occurs in aqueous acid solution with a reported sensitivity of 0.04  $\mu\text{g./ml}$ . Rubin and Knott (26) used a fluorometric procedure to determine phenylephrine in serum. Winefordner (81) determined phenylephrine by its phosphorescent properties at liquid nitrogen temperatures in ethanolic solutions. The wavelengths of excitation are 290 and 240 nm. with phosphorescence

TABLE 6.32-1

<u>System</u>	<u>Rf x 100</u>	<u>Method of Detection</u>	<u>Reference</u>
I	5	spray 1% I <sub>2</sub> in methanol and/or dragendorff's reagent	58
II	21	"	58
III	33	"	58
IV	60	"	58
V	45	"	58
VI	--	potassium ferricyanide 0.6% w/v in 0.5% w/v NaOH quan. UV. densitometry	59
VII	50	ninhydrin	60

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	<u>Silica Gel Plates Coated with</u>	<u>Developing Solvent</u>
I	0.1 M NaOH	Cyclohexane/benzene/diethylamine 75:15:10 (v/v)
II	0.1 M NaOH	methanol
III	0.1 M NaOH	acetone
IV	0.1 M KHSO <sub>4</sub>	methanol
V	0.1 M KHSO <sub>4</sub>	95% ethanol



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TABLE 6.32-1 (continued)

	<u>Silica Gel Plates</u> <u>Coated with</u>	<u>Developing Solvent</u>
VI	cellulose 250 $\mu$	<i>n</i> -butanol/acetic acid/water 4:1:5 v/v organic phase as the developing solvent
VII	silica gel G	<i>n</i> -butanol/acetic acid/water 12:1:7 v/v organic phase as the developing sol- vent

TABLE 6.33-1

<u>Column Support</u>	<u>Stationary Phase</u>	<u>Mobile Phase</u>	<u>Other Compounds Present Not Interfering</u>	<u>Reference</u>
celite 545	acetic acid NaCl (sat'd)	chloroform wash then ether elution	codeine dextromethorphan phenylpropanolamine HCl chlorpheniramine pheniramine pyrilamine doxylamine succinate tripelennamine citrate phenyltoloxamine dihydrogen citrate aspirin	67
celite 545	pH 5.8 buffer pH 5.1 buffer	chloroform wash then elute with 2.4% v/v DEHP in chloro- form	phenylpropanolamine HCl dextromethorphan HBr glyceryl guaiacolate acetaminophen chlorpheniramine phenyltoloxamine citrate aspirin phenolphthalein pyrilamine maleate sulfoxazole brompheniramine maleate	68

TABLE 6.33-1 (continued)

<u>Column Support</u>	<u>Stationary Phase</u>	<u>Mobile Phase</u>	<u>Other Compounds Present Not Interfering</u>		<u>Reference</u>
celite 545 acid washed	sodium borate	chloroform wash then acetylate and elute acety- lated phenyl- ephrine with chloroform saponify	codeine sulfate methapyrilene HCl pyrilamine maleate d-amphetamine sulfate		69
celite 545 acid washed	sodium borate	same as above	codeine phosphate chlorpheniramine maleate		70
celite 545 acid washed	various acids and bases	chloroform wash elute with ethanol	chlorpheniramine maleate pyrilamine maleate codeine phosphate phenylpropanolamine HCl		71

Method of Quantitation - U.V.

TABLE 6.34.1

<u>Column Conditions</u>	<u>Instrumental Conditions</u>	<u>Derivative</u>	<u>Reference</u>
8 ft., 3 mm. I.D., SE-30 1.15% on gas chrom-P 100-140 mesh	col. temp. 135°C, flow rate 30 ml./min. inlet pres. 31 psi	base acetone butanone	72
6 ft., 3 mm. I.D., QFI-0065 (Dow Corning) 2.8% on chromsorb 60-80 mesh	col. temp. 135°C, inlet pres. 30 psi $\beta$ -ionization detector	acetone	72
2 M glass, 4 mm. I.D., 0.1% silicone oil (DC-710) on 60-80 mesh dimethyl-dichlorosilane treated glass beads	inj. 300°C, detc. 260°C flame ionization detc. helium/hydrogen/air flow rate 80/80/450 ml/min., resp. program col. 100°C to 200°C at 10°C/min.	trifluoro- acetic acid	73 <sup>a</sup>
6 ft., 4 mm. I.D., 10% F-60 (methyl polysiloxane) on gas chrom-P 80-100 mesh	detc. 300°C flame ionization temp. program 100°C-200°C @ 1.5°C/min. nitrogen 12 psi air 40 psi, hydrogen 20 psi	HMDS (hexamethyldisilazane) acetone cyclobutanone	74

a = assayed tablets and syrups

TABLE 6.35-1

<u>Resin</u>	<u>Type</u>	<u>Mobile Phase</u>	<u>Method of Quan.</u>	<u>Compounds Present Not Interfering</u>	<u>Reference</u>
Amberlite IR-45	weakly basic	75% ethanol	titration	codeine phos- phate potassium guaiacol- sulfonate camphor menthol	75
Dowex 50-X-1	sulfonic acid	water wash then elute	azo coupling	APAP	39
Dowex 50-X-2	H <sup>+</sup> form	with 0.5 N HCl	Millon's reagent	thyryldiamine HCl	
Dowex 50-X-8				caffein	
Dowex 50-X-12					
Dowex 50-X-16					
Dowex 50-W-X-1					
Amberlite IR 120					

TABLE 6.35-1 (continued)

<u>Resin</u>	<u>Type</u>	<u>Mobile Phase</u>	<u>Method of Quan.</u>	<u>Compounds Present Not Interfering</u>	<u>Reference</u>
Type A	poly- styrene sulfonic acid $\text{NH}_4^+$ form	gradient pH 10-12 or 0.15 $M$ to 0.37 $M$ $\text{NH}_4\text{OH}$	U.V.	metanephrine <i>p</i> -synephrine + 13 other com- pounds	76
AG 50W-X-4	strong cation	1 $N$ HCl in 50% methanol	U.V.	codeine phosphate 77 chlorpheniramine 78 maleate promethazine HCl methapyrilene HCl dextromethorphan HBr	
Alginic Acid	cation	0.01 $N$ HCl	U.V.	pyrilamine maleate 79 codeine phosphate acetaminophen	

occurring at 390 nm.

#### 6.5 Other Methods of Analysis

A non-aqueous titration of phenylephrine to a crystal violet end point using perchloric acid in dioxane-acetic acid medium has been reported (82).

Bromination has been used to determine phenylephrine using bromine water (83) and coulometrically generated bromine (84).

The increase in blood pressure of both rats and guinea pigs is the basis of a biological assay of phenylephrine (85). Salicylamide, *N*-acetyl-*p*-aminophenol and chlorpheniramine maleate do not interfere. Sample sizes of 1.5 to 80 µg. have been determined.

Interference refractometry (86) has been used as a quantitative micro method of phenylephrine analysis.

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## **TOLBUTAMIDE**

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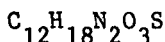
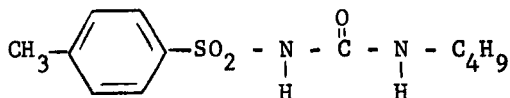
## TOLBUTAMIDE

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- 6.8. Paper Chromatographic
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## 1. Description

### 1.1. Name, Formula, Molecular Weight

Tolbutamide is 1-Butyl-3-(p-tolylsulfonyl)urea<sup>1</sup>. It is also referred to as: N-(4-methyl-benzenesulphonyl)-N'-n-butyl-urea<sup>2</sup>, tolylsulfonylbutylurea<sup>3</sup>, 3-(p-tolyl-4-sulfonyl)-1-butylurea<sup>3</sup>, N-(sulfonyl-p-methylbenzene)-N'-n-butylurea<sup>3</sup>. The most commonly used trade marks are Orinase and Rastinon; 14 additional are listed in the Merck Index 8th Edition<sup>3</sup>. Tolbutamide is a sulfonamide but it is not a sulfanilamide derivative.



Mol. Wt. 270.35

### 1.2. Appearance, Color, Taste, and Odor

Tolbutamide is a white, or practically white, crystalline powder. It has a slightly bitter taste and is practically odorless<sup>1</sup>.

## 2. Physical Properties

### 2.1. Solubility

Practically insoluble in water but forms water-soluble salts with alkalis. It is soluble in alcohol and in chloroform<sup>1</sup>. It is soluble to the extent of 7.8 mg/ml in toluene and 4.4 mg/ml in ethyl acetate:heptane (1:3)<sup>4</sup>.



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### 2.2. Melting Range

The melting range of tolbutamide has been reported as 126-132°<sup>1</sup> and 128.5-129.5°<sup>3</sup>.

### 2.3. Crystal Properties

#### 2.31. Crystal Morphology<sup>5</sup>

##### 2.311. System and Class

Orthorhombic, rhombic pyramidal,

MM2

##### 2.312. Axial Ratio

a:b:c = 0.4504:1:0.3864

##### 2.313. Interfacial Angles

$(011) \wedge (\bar{1}20) = 138^\circ$ ;  $(101) \wedge (10\bar{1}) = 81^\circ$ ;  $(011) \wedge (\bar{1}20) = 96^\circ$

##### 2.314. Habit

Tabular {010} with {101}, {011},  $\{\bar{1}20\}$ . Supplementary twinning is common, but usually with the composition plane and reentrant angles visible.

#### 2.32. Optical Properties<sup>5</sup>

##### 2.321. Refractive Indices (5893A)

$N_x = 1.544$ ;  $N_y = 1.550$ ;  $N_z = 1.604$ ; geometric mean = 1.562

##### 2.322. Molecular Refraction

Observed = 69.4; calculated = 70.4

##### 2.323. Optic Axial Angle (5893A)

$2V = 38^\circ$  calculated from refractive indices;  $42^\circ$  using Mallard's constant.

##### 2.324. Dispersion

$r > v$ , strong.

2.325. Optic Orientation  
a = Y; b = X

2.326. Common Crystal Orientation  
(010) showing centered obtuse bisectrix interference figure.

2.327. Optic Sign  
Positive

2.33. Fusion Properties  
When tolbutamide is melted and slowly cooled, an unstable form crystallizes which, upon reheating, slowly undergoes a solid-solid phase transformation to the stable form<sup>5</sup>.

2.34. X-ray Diffraction  
Precession and Weissenberg photographs of the X-ray diffraction pattern of single crystals of USP Tolbutamide were made in a study to update the powder diffraction file<sup>6</sup>. The crystals were found to be orthorhombic, and systematic absences uniquely determined the space group as P nma. The unit cell parameters measured were in good agreement with the original determination of Shell<sup>5</sup>. The labeling of the unit cell axes given by Shell<sup>5</sup> has, however, been permuted to agree with the International Tables for X-ray Crystallography convention for space group P nma. The new cell dimensions are: a = 20.14Å; b = 9.07Å; and c = 7.78Å.

The DIFMAX computer program was used to arrive at the indexed reflections, 2  $\theta$  values, and d spacings of Table I. In space group P nma, the following conditions limit possible reflections:

o, k, l: k + l must be even

h, k, o: h must be even

Accordingly, deletions of systematically absent reflections were made in Table I.<sup>6</sup>

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TABLE IPossible X-ray Diffraction Maxima for Tolbutamide

H	K	L	2 Theta	D
2	0	0	8.78	10.0608
1	0	1	12.19	7.2507
2	1	0	13.13	6.7332
2	0	1	14.38	6.1510
0	1	1	15.00	5.8998
1	1	1	15.63	5.6614
2	1	1	17.40	5.0893
3	0	1	17.44	5.0780
4	0	0	17.61	5.0304
0	2	0	19.57	4.5308
3	1	1	20.02	4.4299
4	1	0	20.17	4.3981
4	0	1	21.01	4.2231
2	2	0	21.48	4.1312
0	0	2	22.86	3.8864
1	2	1	23.12	3.8423
4	1	1	23.21	3.8278
1	0	2	23.28	3.8159
2	2	1	24.37	3.6480
2	0	2	24.53	3.6253
5	0	1	24.89	3.5737
1	1	2	25.30	3.5168
3	2	1	26.33	3.3807
4	2	0	26.45	3.3666
2	1	2	26.45	3.3659
3	0	2	26.48	3.3627
6	0	0	26.55	3.3536
5	1	1	26.79	3.3245
3	1	2	28.28	3.1526
6	1	0	28.35	3.1451
4	2	1	28.87	3.0893
6	0	1	28.96	3.0792
4	0	2	29.00	3.0755
0	2	2	30.26	2.9499
1	2	2	30.60	2.9187
6	1	1	30.63	2.9155

TABLE I  
(Continued)

H	K	L	2 Theta	D
4	1	2	30.66	2.9123
2	3	0	30.87	2.8930
2	2	2	31.57	2.8307
0	3	1	31.75	2.8154
5	2	1	31.86	2.8059
5	0	2	31.98	2.7956
1	3	1	32.06	2.7883
2	3	1	33.00	2.7113
3	2	2	33.14	2.7002
7	0	1	33.19	2.6960
6	2	0	33.20	2.6955
5	1	2	33.51	2.6713
3	3	1	34.51	2.5960
4	3	0	34.60	2.5896
7	1	1	34.68	2.5841
1	0	3	34.88	2.5697
6	2	1	35.20	2.5467
4	2	2	35.23	2.5446
6	0	2	35.31	2.5390
8	0	0	35.66	2.5152
2	0	3	35.75	2.5091
0	1	3	36.01	2.4911
1	1	3	36.30	2.4722
4	3	1	36.53	2.4568
6	1	2	36.72	2.4448
8	1	0	37.05	2.4235
2	1	3	37.14	2.4181
3	0	3	37.16	2.4169
8	0	1	37.54	2.3930
5	2	2	37.77	2.3791
1	3	2	37.95	2.3683
3	1	3	38.51	2.3352
2	3	2	38.76	2.3206
7	2	1	38.83	2.3169
8	1	1	38.88	2.3137
7	0	2	38.93	2.3110
5	3	1	39.00	2.3069
4	0	3	39.06	2.3034
0	4	0	39.75	2.2654

Figure 1 gives the X-ray powder diffraction pattern for USP Tolbutamide obtained with a General Electric XRD-5 Diffractometer using  $\text{CuK}\alpha$  1, 50 KVP, 16 MA, and tray mount<sup>6</sup>.

#### 2.4. Infrared Spectrum

Tolbutamide can be identified by means of its infrared spectrum (Figure 2). The spectrum of USP Tolbutamide<sup>7</sup> was obtained from a Nujol mull using a Perkin-Elmer Model 421 spectrophotometer. The principal peaks are assigned as follows: 3320, 3190 (urea, NH stretch), 2920, 2850 (alkane, CH stretch including Nujol), 1700, 1660 (urea, C=O stretch), 1600, 1500 (aromatic, C=C stretch), 1555 (urea, amide II), 1460, 1375 (alkane, CH deformation including Nujol), 1335, 1160 (sulfonamide, S=O stretch), and  $815\text{ cm}^{-1}$  (aromatic, CH deformation for para substitution). Other peaks occur at 1320, 1310, 1250, 1220, 1190, 1120, 1095, 900, 740, 725, and  $660\text{ cm}^{-1}$ .

#### 2.5. Nuclear Magnetic Resonance Spectrum

The nuclear magnetic resonance (NMR) spectrum of tolbutamide was obtained using a Varian instrument Model A-60 D. Figure 3 gives the spectrum<sup>8</sup>. The tolbutamide was dissolved in deuterio chloroform with tetramethylsilane as the internal reference. The NMR proton spectral assignments are given in Table II<sup>8</sup>.

#### 2.6. Mass Spectrum

Tolbutamide mass-spectral data are given in Table III<sup>9</sup>. The data were obtained using an Atlas CH4 instrument. The loss of 64 mass units from the molecular ion is attributed to the loss of  $\text{SO}_2$ , a unique loss of these elements from the middle of the tolbutamide molecule. The molecular ion was observed at  $m/e$  270. At 70 ev the most intense peak was observed at  $m/e$  91 (15.4% of total ionization). This peak can be represented as a  $\text{C}_7\text{H}_7^+$  ion which yields  $\text{C}_5\text{H}_5^+$  ( $m/e$  65; metastable at 46.4) upon expulsion of an acetylene molecule after decomposition.

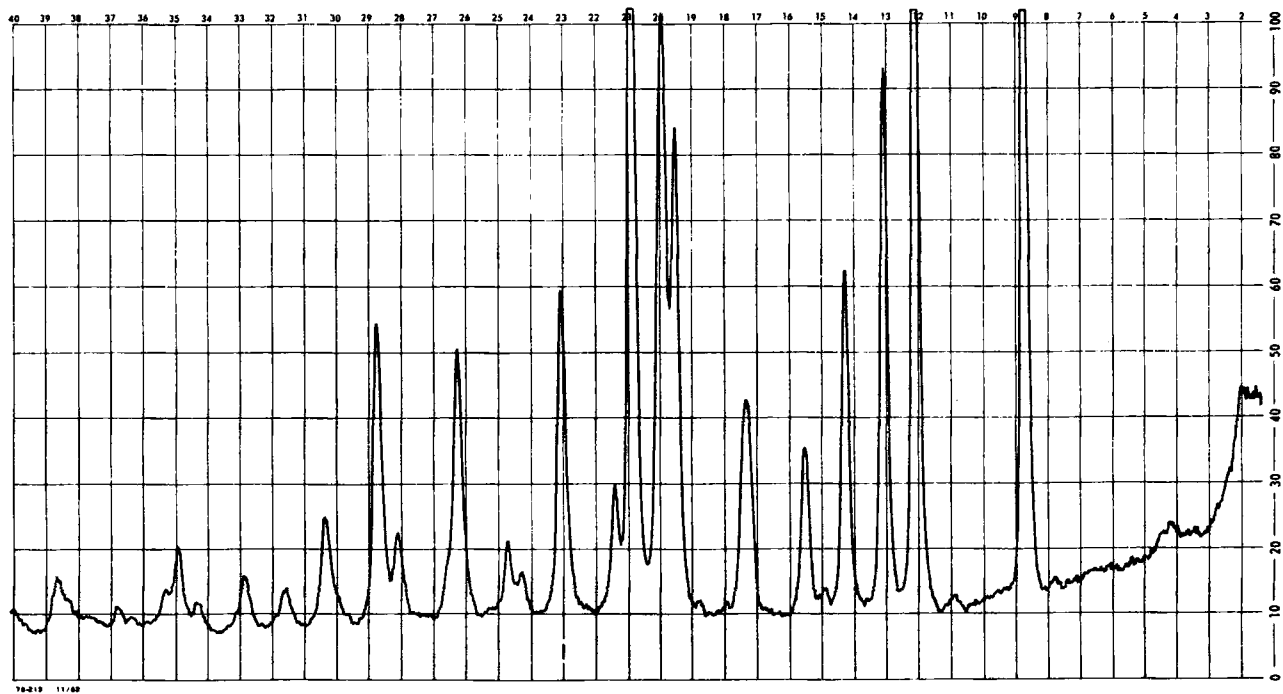


Figure 1. X-ray diffraction pattern of tolbutamide

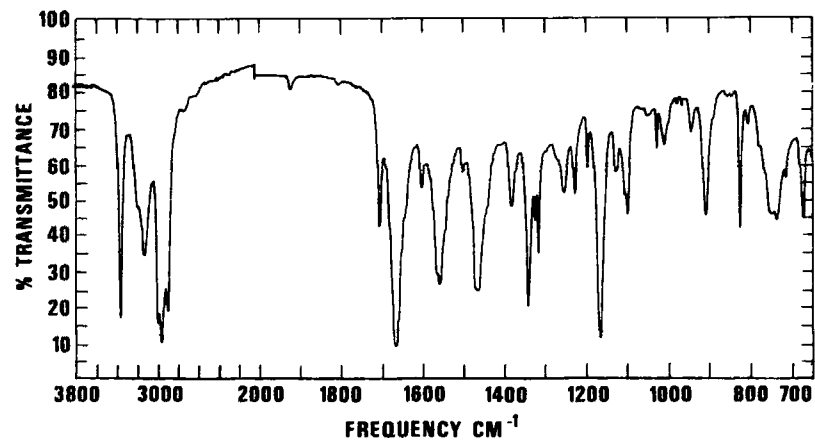


Figure 2. Infrared spectrum of tolbutamide

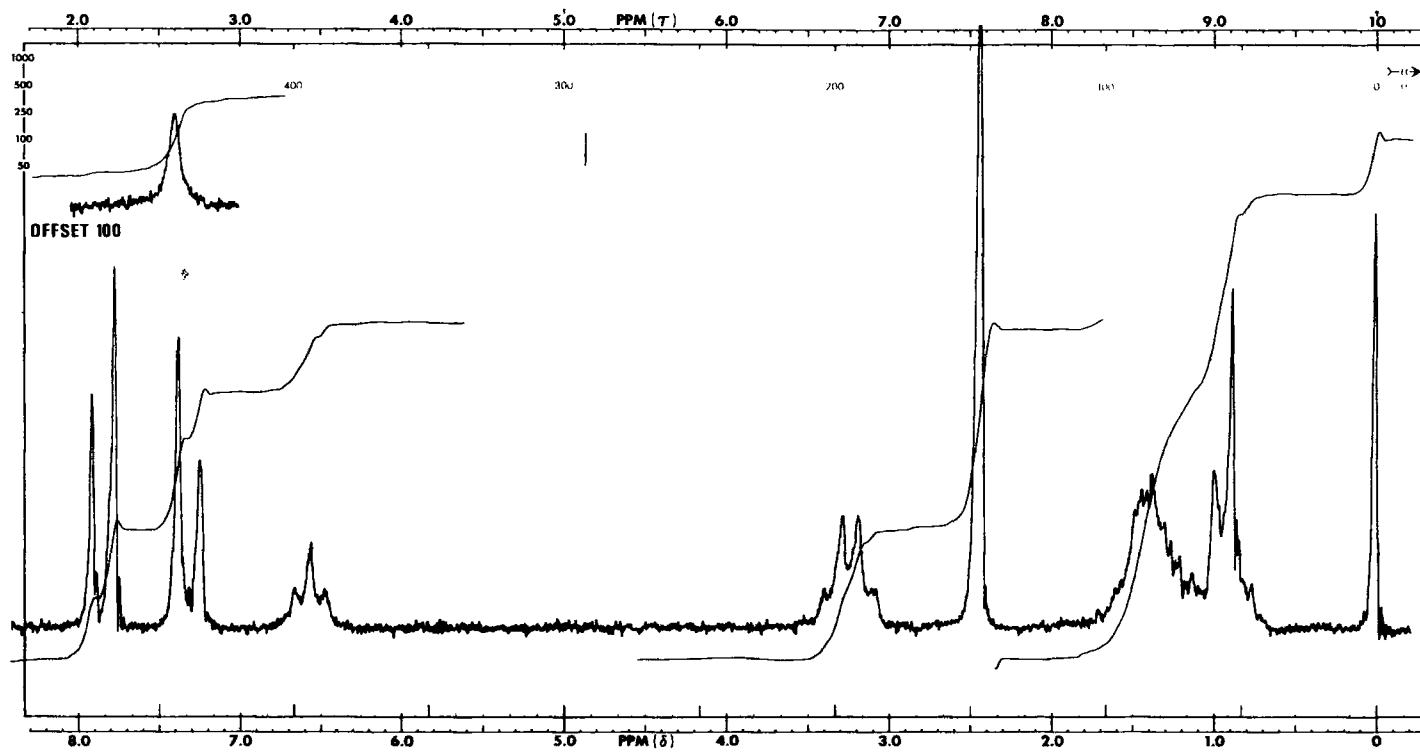
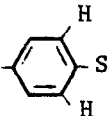
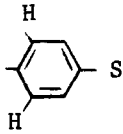


Figure 3. Nuclear magnetic resonance spectrum of tolbutamide



TOLBUTAMIDE

TABLE II  
NMR Spectral Assignments for Tolbutamide

<u>Group</u>	<u>Shape</u>	<u>Chemical Shift</u>	<u>J(Hz)</u>
<u>CH<sub>3</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-</u>	Distorted Triplet	0.88	6.5
CH <sub>3</sub> - <u>CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-</u>	Broad Multiplet	1.40 (approx.)	
<u>CH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>-</u>	Singlet	2.43	
CH <sub>3</sub> -CH <sub>2</sub> -CH <sub>2</sub> - <u>CH<sub>2</sub>-</u>	Quartet	3.23	6.0 (av.)
- <u>NH</u> -CH <sub>2</sub> -	Triplet	6.57	6.0 (av.)
CH <sub>3</sub> - 	Apparent Doublet	7.33	8.0 (av.)
CH <sub>3</sub> - 	Apparent Doublet	7.83	8.0 (av.)
-O <sub>2</sub> S- <u>NH</u> -CO-	Broad Singlet	9.7	

2.7. Ultraviolet Spectrum

The UV spectrum of USP Tolbutamide is shown in Figure 4. The spectrum was obtained with a Cary 15 spectrophotometer using a 15 mcg/ml solution of tolbutamide in anhydrous ethanol. The spectrum, obtained with a 1-cm cell, shows  $\lambda$  max at 228 nm.

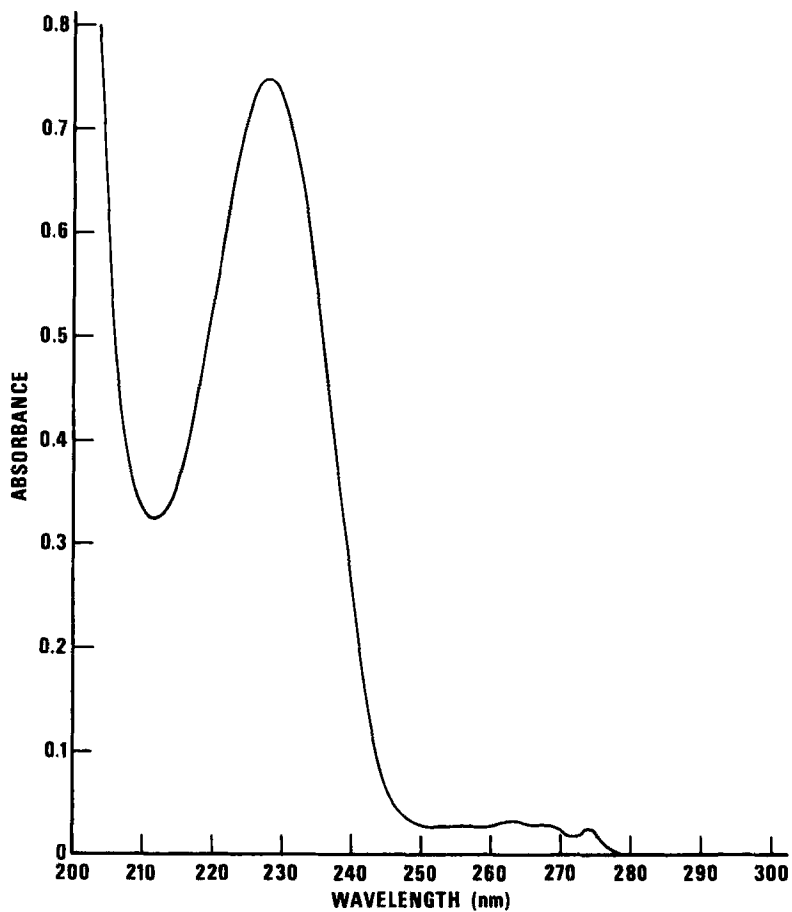


Figure 4. Ultraviolet spectrum of tolbutamide

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TABLE III

Mass Spectral Data for Tolbutamide

<u>m/e</u>	<u>% total ionization</u>	
	<u>70 ev</u>	<u>19 ev</u>
270	2.2	7.5
255	0.2	
241	0.3	
227	1.1	
215	0.4	
206	6.5	35.7
184	0.2	
171	0.6	0.5
163	0.3	
155	7.1	0.4
139	0.6	
115	2.0	1.5
108	14.0	19.4
107	5.3	11.5
99	3.2	3.3
91	15.4	0.1
73	3.2	3.1
72	2.3	0.5
65	4.6	
30	12.6	5.7

## 2.8. pKa

The pKa' of tolbutamide by two separate procedures was 5.43 at 25°C and 5.32 at 37.5°C<sup>10</sup>.

## 2.9. Differential Scanning Calorimetry

The absolute purity of tolbutamide can be determined using differential scanning calorimetry. The purity of Tolbutamide USP Reference Standard with this technique was 99.8%<sup>11,12</sup>. A Perkin-Elmer Differential Scanning Calorimeter Model 1-B was used at a scan speed of 1.25°/min. at a sensitivity of 2 m cal/sec full scale.

W. F. Beyer and E. H. Jensen

### 3. Synthesis

The patent procedure for tolbutamide<sup>2</sup> gives the following example for its preparation: 50 gms of n-butyl isocyanate are stirred at RT into a suspension of 96 gms of sodium 4-methyl-benzenesulfamide in 120 ml of dry nitrobenzene, and the mixture is then heated for 7 hours at 100°C. After being cooled, the reaction mixture, which is a thick magma, is diluted with methylene chloride or ethyl acetate, and the sodium salt of the sulfonylurea formed is separated by centrifuging. The centrifuged crystalline residue freed from organic solvents is dissolved in 500-600 ml of water heated at 50°C and decolorized with charcoal. The precipitate obtained by acidification with dilute hydrochloric acid is dissolved in an equivalent quantity of dilute ammonia solution (about 1:20), again treated with charcoal and reprecipitated with dilute hydrochloric acid. In this manner tolbutamide is obtained in analytically pure form in a yield of 70-80 percent of theory. Menzer et al<sup>13</sup> list p-tolylsulfonamide and p-tolylsulfonylurea as the primary impurities to be expected in the synthesis of tolbutamide.

### 4. Stability

Because of the absence of p-amino groups, which are common to antibacterial sulfonamides, tolbutamide cannot be acetylated. Its p-methyl group, however, renders tolbutamide susceptible to oxidation, occurring chiefly in biological systems.

Thermal decomposition of tolbutamide has been reported by Menzer et al<sup>13</sup>, with reformation of p-tolylsulfonamide and synthesis of butylisocyanate. The latter then reacts with unconverted butylamine and ammonia to form N,N-di-butylurea and N-butylurea. The authors also isolated four additional by-products of tolbutamide, one identified as p-tolylsulfonylbiuret.

The hydrolysis of tolbutamide in an acid environment was investigated by Vogt<sup>14</sup> and in alkaline solution by Haussler and Hajdu<sup>15</sup>. A quantitative dissociation of tolbutamide to p-toluenesulfonamide and n-butyl isocyanate was reported by Ulrich and Sayigh<sup>16</sup> to take place in inert solvent at 160 to 180°C. A significant degradation

occurred in some o/w creams when the drug was dissolved in the oil phase of the emulsion at 70 to 80°C<sup>17</sup>. No such loss occurred when the sulfonylurea was incorporated in the base at room temperature. The authors concluded that the instability of tolbutamide in the oil phase of the emulsion was due to components containing hydroxyl groups. The investigations were expanded to study the dissociation of tolbutamide at 80°C in twelve primary alcohols and in polyethylene glycol 400<sup>18</sup>. Tolbutamide was shown to dissociate to give butylamine and p-toluenesulfonyl isocyanate. N-(p-toluenesulfonyl) carbamate, formed by the reaction of the sulfonylisocyanate with the alcohols, was present in the equilibrium mixture. Bottari et al<sup>19</sup>, in studies investigating the reaction products of tolbutamide and other N-substituted sulfonylureas in alcohols, water, and amines, concluded that dissociation, rather than solvolysis, was the most likely mechanism by which sulfonylureas undergo breakdown at relatively low temperatures. A report by Chubb and Simmons<sup>20</sup> indicates that tolbutamide reacts with refluxing methanol to form the butylamine salt of methyl p-tolylsulfonylcarbamate. They subscribe to a mechanism of methanolysis for the reaction rather than one of pyrolysis.

##### 5. Drug Metabolites

Oxidation of tolbutamide through its p-methyl group appears to be the principal manner of degradation of tolbutamide in man. The p-methyl group is oxidized to form a carboxyl group, converting tolbutamide into its principal metabolite, 1-butyl-3-p-carboxyphenylsulfonylurea (carboxytolbutamide)<sup>21,22</sup>.

The tolbutamide metabolite is highly soluble over the critical acid range of urinary pH values, and its solubility increases with an increase in pH. The measured solubility at pH 5 is 2.8 mg/ml, increasing to 20 mg/ml at pH 5.5; at pH 6.0 by extrapolation, the solubility becomes 300 mg/ml<sup>23</sup>. At 37.5°C and at various pH ranges the following carboxytolbutamide:tolbutamide solubility ratios were determined: pH 5.0, 13:1; pH 5.5, 50:1; pH 6.0, 350:1<sup>10</sup>. A  $pK_a$  of 3.54 at 37.5°C has been determined for the metabolite<sup>10</sup>.

The amount of metabolite in urine can be determined by measuring the color found when amyl acetate-extracted urine is added to 0.1% dinitrofluorobenzene and heated at 150°24.

## 6. Methods of Analysis

### 6.1. Elemental Analysis of tolbutamide<sup>4</sup>.

<u>Element</u>	<u>% Theory</u>	<u>% Reported</u>
Carbon	53.31	53.43
Hydrogen	6.71	6.99
Nitrogen	10.36	10.29
Sulfur	11.86	11.88

### 6.2. Phase Solubility Analysis

Phase solubility profiles of tolbutamide were obtained with solvent systems of toluene and ethyl acetate:heptane (1:3), giving solubilities of  $7.79 \pm 0.15$  mg/gm and  $4.41 \pm 0.08$  mg/gm respectively<sup>4</sup>. The calculated purity of the tolbutamide sample based on its solubility profile in toluene was  $100.1 \pm 0.70\%$  and in ethyl acetate:heptane (1:3) it was  $99.6 \pm 0.41\%$ .

### 6.3. Titrimetric

The procedure of Franchi<sup>25</sup> depends on titration of tolbutamide with sodium methoxide in anhydrous pyridine-chloroform-methanol.

In a reported titrimetric method of assay for tolbutamide in non-aqueous media<sup>26</sup>, 50 to 150 mg is dissolved in 10 ml of anhydrous acetone or pyridine and titrated with 0.1N sodium methoxide to a phenolphthalein endpoint. In a mixture of benzene and methanol (2:1), thymol blue can be used as indicator. A similar procedure was reported by Dave and Patel<sup>27</sup>.

Simionovici and Conu<sup>28</sup> developed a direct titration method whereby approximately 300 mg of

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tolbutamide is dissolved in 25 ml of acetone previously neutralized to cresol red and titrated with 0.1N sodium hydroxide to a rose-violet color.

In a procedure depending on hydrolysis, the previous authors<sup>28</sup> treated approximately 300 mg of tolbutamide with 10 ml of ethanediol and 2 ml of conc. hydrochloric acid, and applied 120-122°C heat for 30 min. The mixture was then diluted with water, treated in a Kjeldahl flask with 15 g sodium hydroxide and the amine was distilled off into 25 ml of 0.1N hydrochloric acid, the excess determined by titration. Assay variation of  $\pm 1.0\%$  was reported.

Parikh and Mukherji<sup>29</sup> developed a titration procedure for tolbutamide in which tolbutamide was converted into its sodium salt, combining it quantitatively with silver nitrate to form an insoluble silver salt. Ferric ammonium sulphate was used as the indicator and 0.05N ammonium thiocyanate as the titrant.

Beyer and Houtman<sup>30</sup> described automated titrimetric procedures for the analysis of tolbutamide tablets using a modified Fischer Titralyzer. The relative standard deviation for the procedure was approximately 1%.

The USP assay procedure<sup>1</sup> for tolbutamide depends upon the titration of the drug in neutralized aqueous alcohol with sodium hydroxide as the titrant, and phenolphthalein as indicator.

### 6.4. Ultraviolet Spectrophotometric

Spingler and Kaiser<sup>31</sup> determined tolbutamide in serum after lyophilization, extraction with acidified ethyl acetate, reduction to dryness, and finally dissolution in methanol. Absorbances at 228 nm and 280 nm were used in quantitating tolbutamide.

Forist et al<sup>32</sup> developed an analytical procedure for the determination of tolbutamide in plasma. The

procedure depends on chloroform extraction of weakly acidified plasma, concentration of the extraction to dryness, dissolution of the dry residue in ethanol, treatment of the solution with charcoal, and measurement of the absorbance of an alcoholic solution at 228 nm. Experiments with human and dog plasma gave recoveries of added tolbutamide of about 98-99%. Refinements in the method were reported by Bladh and Norden<sup>33</sup>, and Delaville and Palazzoli<sup>34</sup>.

A procedure for the automated analysis of tolbutamide tablets has been reported using Technicon Corporation's AutoAnalyzer equipment<sup>35</sup>. The analysis was carried out at a wavelength of 263 nm at a sampling rate of 20/hour. A coefficient of variation of approximately 1% was obtained.

The USP XVIII<sup>1</sup> procedure for tolbutamide tablets depends upon the UV absorbance of extracted tablets in chloroform at a wavelength of 263 nm. A UV dissolution rate test for tolbutamide tablets is described in the 1st supplement to the USP XVIII using tris(hydroxymethyl) aminomethane buffer at pH 7.6 and a stirring rate of 150 rpm. Filtered samples are read at a wavelength of 226 nm. The test specifies that the time required for 50% of the labeled amount of tolbutamide in tablets to dissolve is not more than 45 minutes.

#### 6.5. Colorimetric

McDonald and Sawinski<sup>36</sup> developed a colorimetric method involving the reaction between tolbutamide, 2-naphthol, sodium nitrite, and concentrated sulfuric acid forming a red color. The method is reported to be applicable over the concentration range of approximately 50 mcg to 10 mg of tolbutamide per ml of solution.

Chulski<sup>37</sup> described a method for the determination of tolbutamide in serum by extracting acidified serum with chloroform. After reducing the chloroform extract to dryness, an alcoholic solution of p-N-dimethylaminobenzaldehyde is added and the solution reduced to dryness. The dry residue is heated for 2-1/2 hours at



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70°C and alcohol is added. Absorbance of the solutions is measured at 395 nm. The average recovery of tolbutamide from serum was reported to be near 100% with a standard deviation of approximately 5%.

The colorimetric determination of serum tolbutamide developed by Spingler<sup>38</sup> depends on the reaction of tolbutamide and dinitrofluorobenzene at 150°C following extraction of acidified serum with amyl acetate. The absorbance is determined at about 380 nm. After a study of the method of Spingler<sup>38</sup>, Pignard<sup>39</sup> suggested improvements that were reported to increase specificity and range of usefulness. Among those suggested were purification of reagents and lengthening the heating time from 5 to 30 minutes at a temperature of 100°C instead of 150°C.

Dorfmueller<sup>40</sup> reported the reaction of tolbutamide in alkaline media with diacetylmonoxime and N-phenylanthranilic acid, followed by acidification and heat and the addition of sodium persulfate and sodium acetate to form a blue color.

Mesnard and Crockett<sup>41</sup> extended the work of Richter<sup>42</sup>, which involved the determination of aliphatic amino acids, to the analysis of tolbutamide in biological fluids. The method is based on the strong yellow coloration of substituted ammonium picrates in selected anhydrous solvents, in which picric acid is essentially colorless. Of the two absorbance maxima observed (355 nm and 412 nm) the maximum at 412 nm was used to avoid absorptive materials in blood and urine that could interfere at the 355 nm wavelength. The authors also reviewed other methods for the determination of tolbutamide and other non-amino hypoglycemic sulfonamides<sup>43,44</sup>.

Kern<sup>45</sup> reported a procedure for the determination of tolbutamide in blood following extraction with ethylene chloride at pH 5. After nitration, diazotization and coupling with N(1-naphtyl) ethylene diamine, an azo dye is produced that is measured at 547 nm.

A number of other investigators have described colorimetric procedures for tolbutamide<sup>46-49</sup>.

#### 6.6. Gas Chromatographic

A gas-liquid chromatographic method for the determination of tolbutamide in blood, urine, and tablets was reported by Sabih and Sabih<sup>50</sup>. A method for blood and urine involved extraction of tolbutamide from acidified plasma or urine and conversion to the methyl derivative with dimethylsulfate in the presence of base. A gas chromatograph (F & M 5755B) with a flame ionization detector and fitted with a stainless steel column was used. The column was packed with diatomaceous earth (Gas chrom Q) and coated with 5% DC-200. Temperatures of 205-210° for the column, 320° for the detector, and 330° for the injection port were used. Additional GLC methods for tolbutamide in blood have been reported by Prescott and Redman<sup>51</sup> and Simmons et al<sup>52</sup> also involving methylation with dimethyl sulfate.

#### 6.7. Liquid Chromatographic

A liquid chromatographic assay procedure for tolbutamide in tablets (also applicable to bulk drug) was described by Beyer<sup>53</sup>. A duPont Model 820 Liquid Chromatograph with an HCP column (stainless steel) 1 M long x 2.1 mm ID and a mobile phase of pH 4.4 monobasic sodium citrate buffer in 15% methanol at a flow rate of 0.36 ml/min were used for the analysis. The relative standard deviation was less than 2% and recovery was quantitative.

#### 6.8. Paper Chromatographic

Chakrabarti<sup>54</sup> reported a paper chromatographic analysis of tolbutamide in various solvent systems. The developed paper is reacted with phenyl hydrazine and sprayed with a solution of ammoniacal Ni<sup>2+</sup> giving pink to violet spots, depending on the concentration of tolbutamide solutions.

Hentrich<sup>55</sup> described a paper chromatographic separation of tolbutamide by chromatographing the butyl amine produced when tolbutamide is reacted with a modified Folin reagent. After heating the paper at 180-200°, a brown spot is produced.

Abdel-Wahab and El-Allawy<sup>56</sup> reported a procedure utilizing paper chromatography for radio isotopes and the determination of tolbutamide. The authors employed various developers and coloring reagents such as ninhydrin. Radio-activation of dried, developed chromatograms, using  $I^{131}$  was applied to determine  $R_f$  values. Investigations of  $S^{35}$  labeled tolbutamide showed that paper chromatography, accompanied by radioscanning or autoradiography could be used for the separation, detection, and determination of tolbutamide.

An infrared identification of tolbutamide in human serum employing paper chromatography was reported by Krivis and Forist<sup>57</sup>. The procedure depends upon extraction of serum with chloroform, eventual reduction to dryness, dissolution in methylene chloride, and application to prewashed Whatman No. 1 paper. After development in a butanol-water-piperidine (81:17:2) system by descending chromatography, the tolbutamide zone was eluted with water. A potassium bromide micropellet prepared from a methylene chloride extraction of the aqueous eluate was then examined by infrared spectrophotometry.

#### 6.9. Thin Layer Chromatographic

Strickland<sup>58</sup> described TLC procedures for the separation and detection of microgram amounts of tolbutamide in the presence of acetohexamide, chlorpropamide, and phenformin HCl. Various solvent systems and spray reagents were used to determine relative  $R_f$  values. A solvent system consisting of acetone-benzene-water (65:30:5) separated tolbutamide and the other three anti-diabetic agents. The limit of detection for tolbutamide using UV was about 1 mcg.

A TLC procedure for the detection of tolbutamide in blood and urine was reported by Baumler and Rippstein<sup>59</sup>. The drug is extracted with ether and chromatographed using Kieselgel-cellulose (1:1) as the TLC support and developed with benzene-methanol (4:1). Tolbutamide appears as a violet spot when the developed plate is sprayed with ninhydrin-stannous chloride and heated, then sprayed with acidified ninhydrin and heated again.

Guven et al<sup>60</sup> identified tolbutamide amongst other anti-diabetic drugs by TLC on silica gel using a solvent system composed of butanol-acetic acid-water (10:2:1). A solution of copper sulfate (10%) ammonia (2%) (5:1) was used to detect the spots, with tolbutamide exhibiting a green color.

A report by Hutzul and Wright<sup>61</sup> for the detection of small amounts of impurities (for example, 0.05% p-toluenesulfonylurea) in tolbutamide, makes use of a "Moscow" method of TLC developed by Mistryukov<sup>62</sup>. The plate, 5x8x1/8" frosted window pane, is covered with adsorbent and maintained horizontally while the developing solution is fed to one edge by capillary action. p-Toluenesulfonylurea is separated from tolbutamide using Davison silica gel as adsorbent, benzene-acetone-methanol-acetic acid (70:20:9:1) as developing solvent, and visualized using mists of hypochlorous acid, ethanol, and an aqueous solution of acetic acid with iodide and saturated with benzidine. For the detection of p-toluenesulfonamide, an adsorbent of aluminum oxide and a developing solvent of benzene-acetone-methanol (70:20:10) was used. Agents for visualization were the same as those for p-toluenesulfonylurea.

Menzer et al<sup>13</sup> used a developing system comprised of chloroform:glacial acetic acid (99:1) and silica gel H plates, 0.25 mm thick to separate tolbutamide from its by-products. Evaluations were made under UV light after spraying with xanthidrol solution. Their work also disclosed a new by-product in the synthesis of tolbutamide: p-tolylsulfonylbiuret. Table IV summarizes their results.

TABLE IV

Relative TLC Rf Values for Tolbutamide and By-Products  
Using a Chloroform:Glacial Acetic Acid (99:1)  
Developing Solution

<u>Compound</u>	<u>Rf</u>
Tolbutamide	0.85
N,N'-Dibutylurea	0.56
P-Tolylsulfonamide	0.47
P-Tolylsulfonylurea	0.26
P-Tolylsulfonylbiuret	0.13
N-Butylurea	0.12

Reisch *et al*<sup>63</sup> reported five TLC developing systems using n-butanol in combination with two to three other organic solvents. Spots were detected on silica gel G plates when sprayed with visualizing agents. TLC was applied by Neidlein *et al*<sup>64</sup> and Glogner *et al*<sup>65</sup> in separating tolbutamide from other sulfonamides.

#### 6.10. Coulometric

A Mercurocoulometric method for the determination of tolbutamide has been reported by Kalinowski and Korzybski<sup>66</sup> and Voicu<sup>67</sup>.

#### 7. Pharmacokinetics and Toxicity

The half-life in human subjects for 1 gm of tolbutamide given as a single dose following an overnight fast is reported by McMahon *et al*<sup>68</sup> to be 5.7 hours. The LD<sub>50</sub> for tolbutamide administered orally to rats is 2,344 mg/kgm and in mice injected IP is 1,232 mg/kgm<sup>68</sup>.

Inactivation of tolbutamide to carboxytolbutamide occurs in man and is rapidly excreted in urine as the principal metabolite.

8. Identification

Identification tests for tolbutamide are given in U.S.P. XVIII<sup>2</sup>. The tests depend upon: a) the infrared adsorption spectrum of a mineral oil dispersion of the drug in the range of 2 to 12  $\mu$ ; b) formation of an orange-red color after the drug is refluxed with dilute sulfuric acid, steam distilled in dilute hydrochloric acid after being made strongly alkaline, made alkaline with acetate and borate buffer, and reacted in an ice bath with p-nitroaniline and sodium hydroxide; and c) production of p-toluenesulfonamide (melting between 136° and 141°) by refluxing in dilute sulfuric acid, cooling the solution, collecting and purifying the crystals.

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This monograph is based to a great extent on a preliminary compilation of analytical information on tolbutamide by Dr. Arlington A. Forist. His background data facilitated the preparation of the monograph. Mrs. Betty Breseman deserves special recognition for preparation of the manuscript in its final form. The authors also wish to acknowledge the valuable secretarial support of Mrs. Marilyn K. Nelson.

**TRIMETHAPHAN CAMSYLATE**

*Kenneth W. Blessel, Bruce C. Rudy, and Bernard Z. Senkowski*

## INDEX

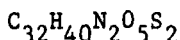
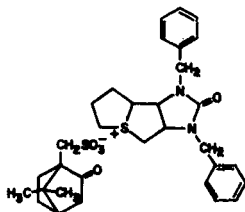
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## TRIMETHAPHAN CAMSYLATE

### 1. Description

#### 1.1 Name, Formula, Molecular Weight

Trimethaphan camsylate is (+)-1,3-dibenzyldecahydro-2-oxoimidazo[4,5-c]thieno[1,2-a]-thiolium 2-oxo-10-bornane sulfonate.



Molecular Weight: 596.81

#### 1.2 Appearance, Color, Odor

Trimethaphan camsylate is a white crystalline powder which is odorless or has a slight odor.

#### 1.3 Isomeric Forms

Trimethaphan camsylate has four possible isomers, grouped in two pairs of enantiomers.

### 2. Physical Properties

#### 2.1 Infrared Spectrum

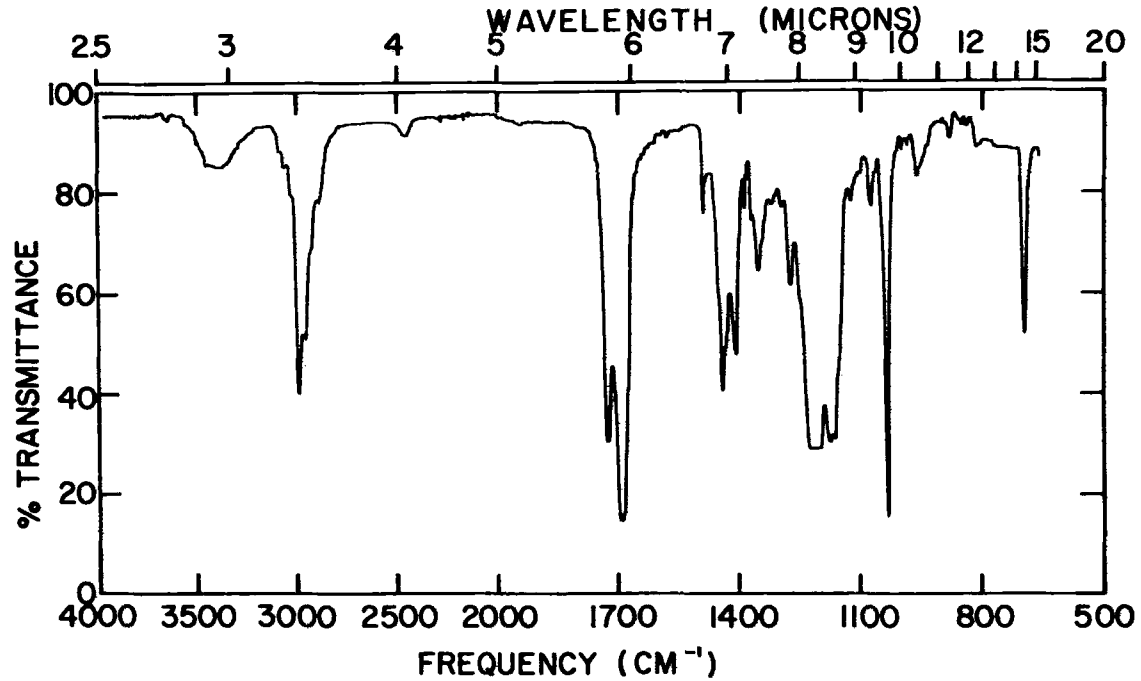
The infrared spectrum of a sample of reference standard trimethaphan camsylate is shown in Figure 1 (1). The spectrum was recorded using a 5% w/v solution in chloroform, utilizing a Perkin Elmer 621 Spectrophotometer equipped with 0.1 mm NaCl liquid cells. The following assignments of some of the bands in the spectrum have been made which are shown in Table I below (1).

Table I

<u>Band</u>	<u>Assignment</u>
1735 $\text{cm}^{-1}$	C=O stretch in the camphor-sulfonic acid moiety
1700 $\text{cm}^{-1}$	C=O stretch of the trimethaphan moiety

Figure 1

Infrared Spectrum of Trimethaphan Camsylate



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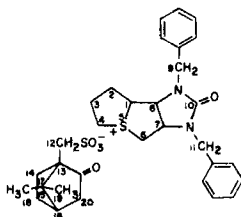
1447 $\text{cm}^{-1}$	$\text{CH}_2$ and $\text{CH}_3$ deformations
701 $\text{cm}^{-1}$	out-of-plane deformations of the monosubstituted benzene rings.

## 2.2 Nuclear Magnetic Resonance Spectrum (NMR)

The NMR spectrum of a sample of reference standard trimethaphan camsylate is shown in Figure 2 (2). The solvent used was  $\text{DMSO-d}_6$  and the concentration of the trimethaphan camsylate was 54.8 mg/0.5 ml. Due to the complex nature of the spectrum, a considerable amount of spin-spin decoupling was necessary in order to obtain the assignments shown in Table II (2). An arbitrary set of numbers was used on the structural formula, shown below, for ease in presenting the assignments.

Table II

NMR Spectral Assignments for Trimethaphan Camsylate

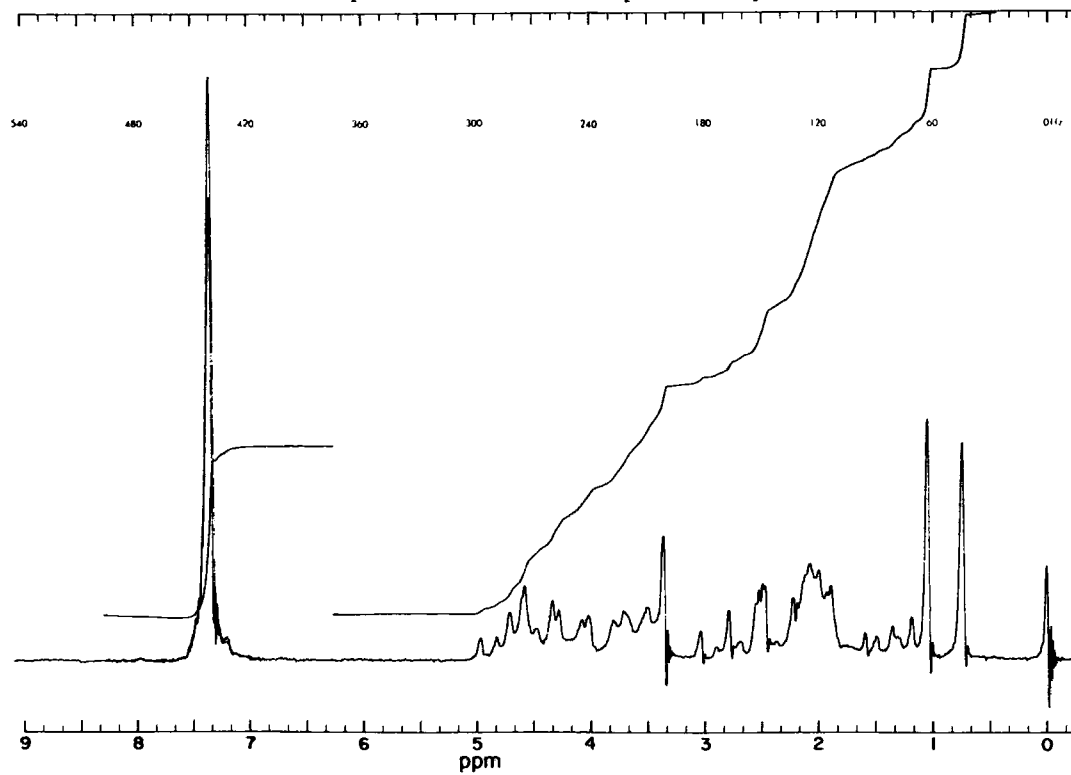


<u>Proton</u>	<u>Total No. of Protons</u>	<u>Chemical Shift (ppm)</u>	<u>Multiplicity</u>	<u>Coupling Constant</u>
$\text{C}_{18}$ protons	3	0.73	Singlet	
$\text{C}_{19}$ protons	3	1.03	Singlet	
$\text{C}_{14}$ and $\text{C}_{15}$ protons	4	1.10-1.60	Multiplet	
$\text{C}_{16}$ and $\text{C}_{20}$ protons	3	$\sim 2.3$	Multiplet	
$\text{C}_{12}$ protons	2	$\sim 3.0$	Triplet	
$\text{C}_9$ and $\text{C}_{11}$ protons	4	4.0-4.3 4.6-5.0	2 pairs of Doublets	$J(\text{C}_9^{\text{H}}\text{C}_{11}^{\text{H}}) = 16 \text{ Hz}$



Figure 2

NMR Spectrum of Trimethaphan Camsylate



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C <sub>1</sub> , C <sub>2</sub> , C <sub>3</sub> , C <sub>4</sub> ,	11	2.0-3.8	Two sets of
C <sub>6</sub> , C <sub>7</sub> and C <sub>8</sub>		4.5-5.9	Multiplets
protons			
aromatic	10	7.34	Singlet
protons			

## 2.3 Ultraviolet Spectrum

The ultraviolet spectrum of reference standard trimethaphan camsylate (a) is shown in Figure 3 (3). The concentration was 1.00 mg/ml in chloroform. The curve shows a maximum at 258 nm ( $\epsilon = 3.9 \times 10^2$ ) having two shoulders on the rising portion of the curve. Also shown on the same figure is the baseline scan (b) and the ultraviolet spectrum of the bromocresol green complex (c) with trimethaphan camsylate which is used for colorimetric determination.

## 2.4 Fluorescence Spectrum

Trimethaphan camsylate (1 mg/ml in methanol) has extremely weak excitation and emission spectra which are shown in Figure 4 (4). The instrument used was a Farrand MK-1 recording spectrofluorometer. Excitation at 290 nm produced an emission spectrum having a maximum at 416 nm.

## 2.5 Mass Spectrum

The low resolution mass spectrum of a sample of reference standard trimethaphan camsylate is shown in Figure 5 (5). The spectrum was obtained with the aid of a CEC 21-110 mass spectrometer at an ionizing energy of 70 eV, interfaced with a Varian data system 100 MS. The data system accepted the output of the spectrometer, calculated the masses, compared the intensities to that of the base peak and plotted the intensities as a series of lines whose heights were proportional to the intensities.

Trimethaphan camsylate is a thiophanium salt and as such has very low volatility, therefore, a highly characteristic mass spectrum cannot be expected. The highest mass observed by low resolution was  $m/e$  488. The base peak was observed at  $m/e$  91, corresponding to  $C_6H_5-CH_2O$ . A high resolution scan showed masses up to  $m/e$  578 which probably arises from the loss of  $H_2O$  from the molecular ion. Other masses were observed at  $m/e$  548, probably due to the loss of SO from the parent mass and

Figure 3

Ultraviolet Spectra

- (a) Trimethaphan Camsylate
- (b) Solvent
- (c) Trimethaphan Camsylate - Bromocresol Green Complex

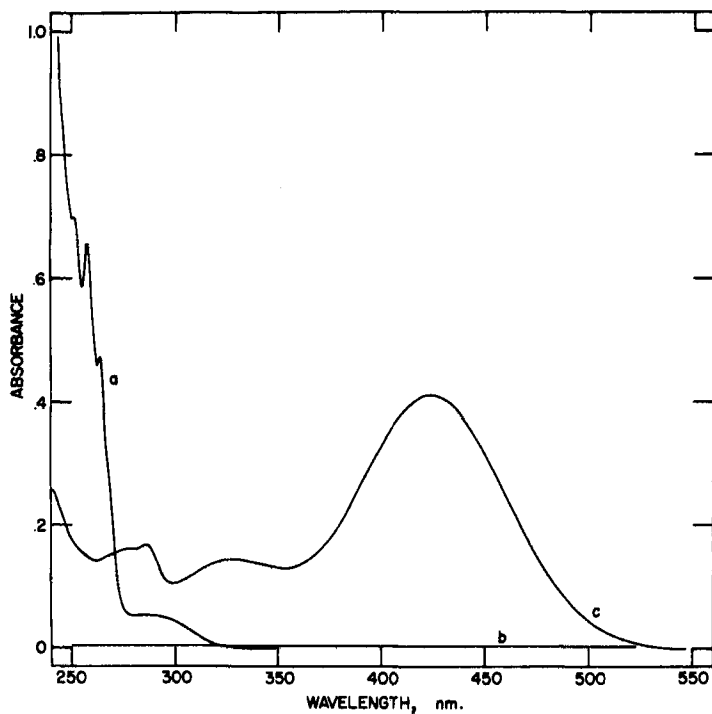


Figure 4

Fluorescence Spectra of Trimethaphan Camsylate

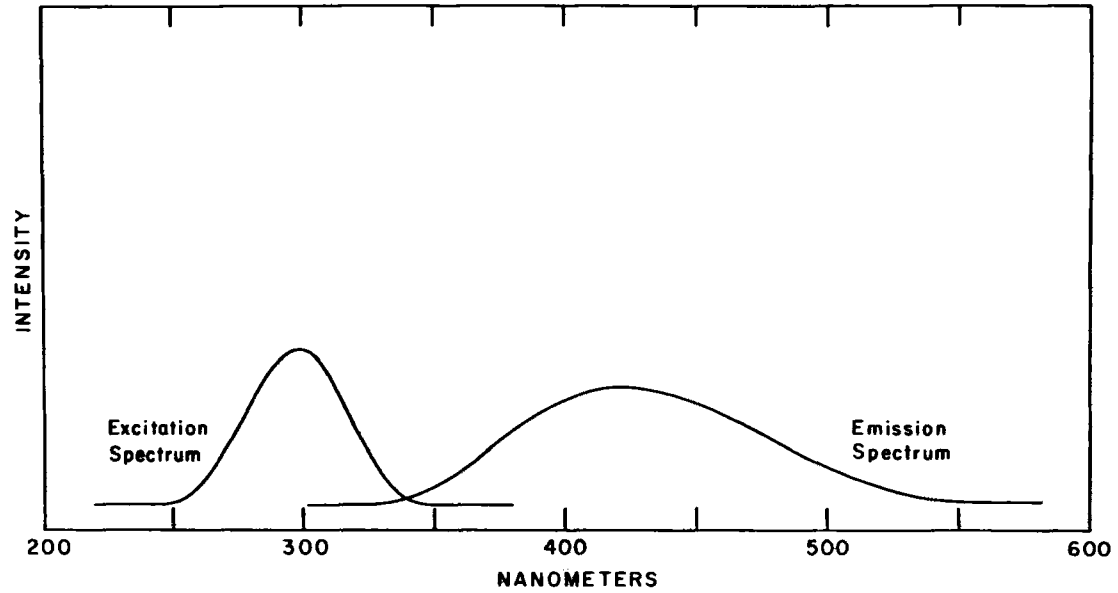
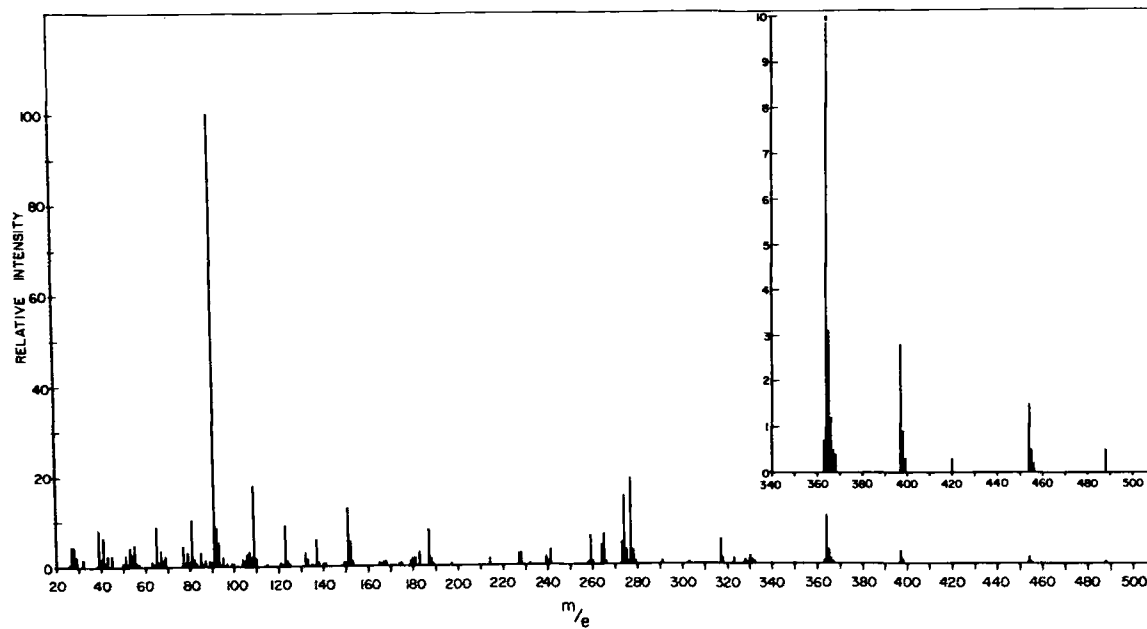


Figure 5

Mass Spectrum of Trimethaphan Camsylate



m/e 545, due to the loss of SH and H<sub>2</sub>O from the molecular ion. These peaks are rather peculiar in that they appear to arise from fragments containing both the acid and base portions of the molecule (5).

## 2.6 Optical Rotation

The value reported for the specific rotation of trimethaphan camsylate in the United States Pharmacopeia XVIII is "not less than +20° and not more than +23° determined in a solution containing 400 mg in each 10 ml" (6). A graph of specific rotation as a function of wavelength is also presented in Figure 6 (7). The data were obtained by converting rotation values from a Jasco ORD-UV 5 instrument to specific rotation. The specific rotation was zero at 294 nm and 225 nm. It can be seen that the specific rotation changes rapidly at wavelengths below 300 nm.

## 2.7 Melting Range

Trimethaphan camsylate melts with decomposition over a range of 230-235°C when a Class Ia procedure is used (6).

## 2.8 Differential Scanning Calorimetry (DSC)

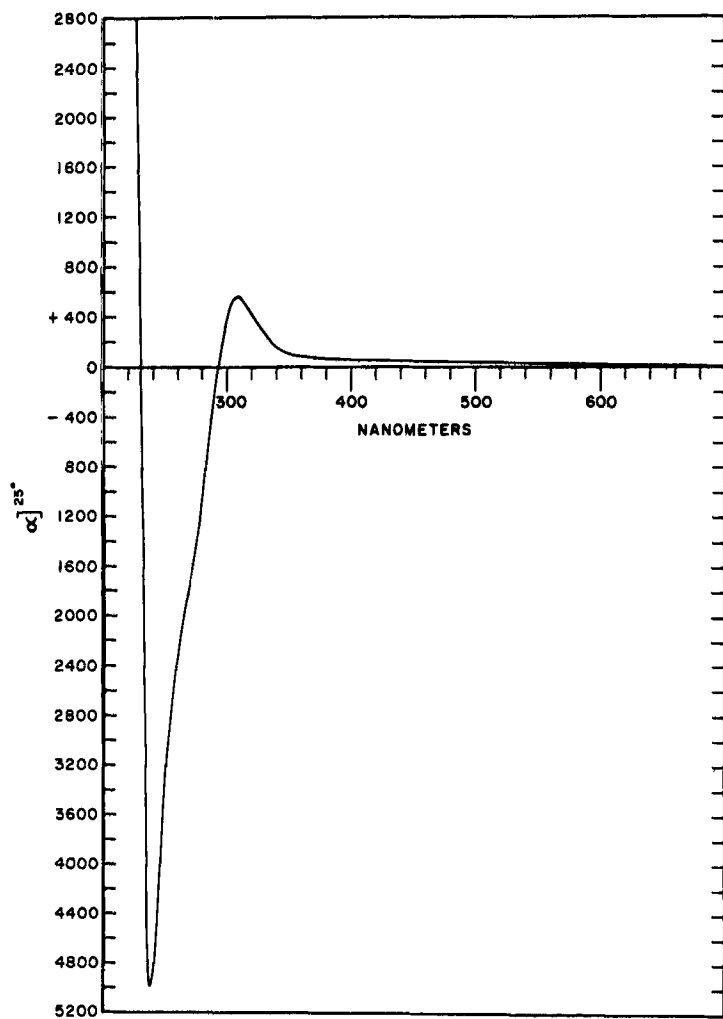
The DSC curve for a sample of reference standard trimethaphan camsylate is shown in Figure 7 (8). This curve was obtained at a scan rate of 10°C/min. in a nitrogen atmosphere utilizing a Perkin Elmer DSC-1B. The extrapolated onset of the melting endotherm, accompanied by decomposition, is  $234.9 \pm 0.1^\circ\text{C}$  and the peak is at  $238.4 \pm 0.4^\circ\text{C}$ . All temperatures have been corrected. Because of the decomposition during the melt, the  $\Delta H_f$  cannot be calculated with much reliability, however, its value is approximately 12 kcal/mole (8).

## 2.9 Thermogravimetric Analysis (TGA)

The TGA curve for reference standard trimethaphan camsylate showed no weight loss from ambient temperature to 265°C at a heating rate of 10°C/min. At about 265°C, weight loss began which amounted to approximately 70% of the weight at 345°C (8).

Figure 6

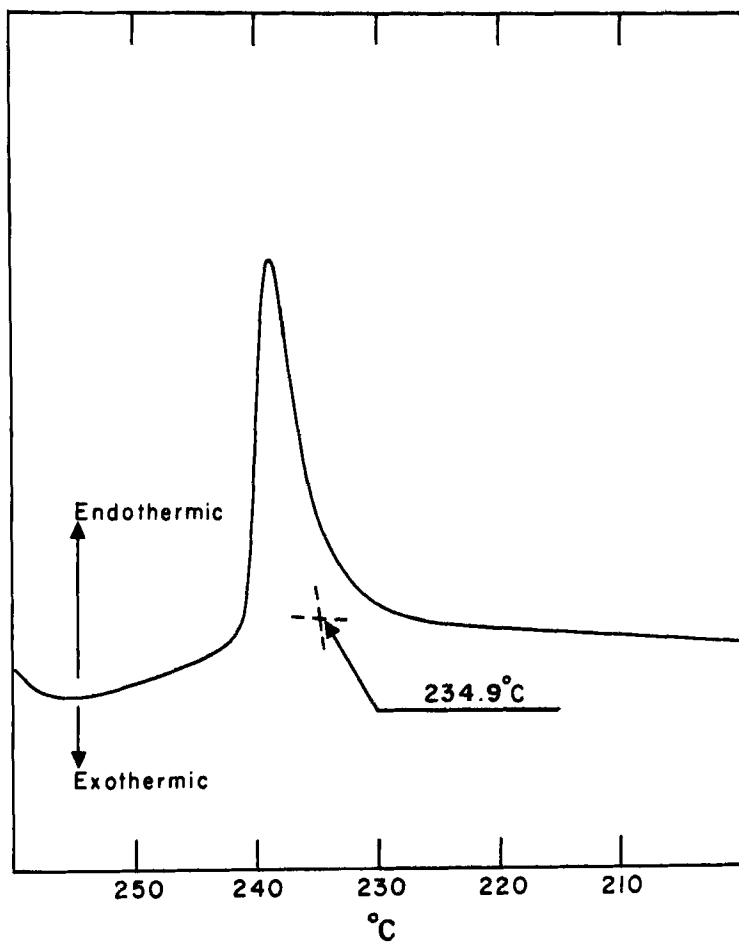
Specific Rotation vs Wavelength for Trimethaphan Camsylate



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Figure 7

DSC Curve of Trimethaphan Camsylate





2.10 Solubility

The solubility data shown in Table III was obtained at 25°C for reference standard trimethaphan camsylate (9).

Table III

## Solubility Data for Trimethaphan Camsylate

<u>Solvent</u>	<u>Solubility (mg/ml)</u>
diethyl ether	0.01
petroleum ether (30-60°)	0.05
2-propanol	20.15
3A alcohol	89.06
chloroform	>500.
95% ethanol	175.80
benzene	2.59
methanol	>500.
water	>500.

2.11 Crystal Properties

The x-ray powder diffraction data for a sample of reference standard trimethaphan camsylate is given in Table IV (10). The operating parameters of the instrument are given below.

Instrumental Conditions

## General Electric Model XRD-6 Spectrogoniometer

Generator:	50 KV, 12-1/2 MA
Tube target:	Cu K $\alpha$ = 1.542 Å
Optics:	0.1° Detector slit
	M.R. Soller slit
	3° Beam slit
	0.0007" Ni filter
	4° take off angle
Goniometer:	Scan at 0.2° 2 $\theta$ per minute
Detector:	Amplifier gain - 16 course, 8.7 fine
	Sealed proportional counter tube and DC voltage at plateau

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Pulse height selection  $E_L$  -  
5 volts  
Eu - out  
Rate meter T.C. 4  
2000 C/S full scale  
Recorder: Chart Speed 1 inch per 5  
minutes  
Samples: Prepared by grinding at room  
temperature.

Table IV

Interplanar Spacings in Trimethaphan Camsylate from Powder  
Diffraction Data

$2\theta$	$d(\text{\AA})^*$	$I/I_0^{**}$	$2\theta$	$d(\text{\AA})^*$	$I/I_0^{**}$
7.04	12.5	100	26.94	3.31	16
10.94	8.09	14	28.06	3.18	18
12.00	7.38	13	28.86	3.09	14
12.26	7.22	19	29.92	2.99	25
12.52	7.07	53	30.42	2.94	2
12.76	6.94	23	31.12	2.87	4
14.14	6.26	52	31.66	2.83	4
14.84	5.97	7	32.54	2.75	8
16.22	5.46	96	34.54	2.60	10
17.54	5.06	81	35.10	2.56	6
18.68	4.75	44	35.86	2.50	3
19.24	4.61	98	36.96	2.43	3
20.78	4.27	57	37.44	2.40	6
21.28	4.18	48	37.84	2.38	4
21.94	4.05	20	38.96	2.31	4
22.34	3.98	21	39.56	2.28	3
22.82	3.90	11	40.40	2.23	4
23.28	3.82	10	41.00	2.20	1
24.50	3.63	25	41.76	2.16	3
24.86	3.58	11	43.00	2.10	4
25.20	3.53	9			
25.58	3.48	13			
25.94	3.43	4			

\*d = (interplanar distance)  $\frac{n\lambda}{2 \sin \theta}$

\*\* $I/I_0$  = relative intensity (based on highest intensity  
of 100)

### 3. Synthesis

Trimethaphan camsylate can be obtained as a by-product in the synthesis of biotin. The synthetic routes to biotin have been reported in several patents (11-13).

### 4. Stability Degradation

A study of the stability of trimethaphan camsylate was carried out by heating the material in the dry form for different periods of time. The solid was dissolved in double distilled water at a concentration of 5% and an estimate of stability was made by noting the degree of turbidity caused by decomposition products. It was observed that on heating at 100°C for periods up to 2 hours, only a slight turbidity was noted in the solution (14). The decomposition of trimethaphan camsylate under extreme conditions was also studied. It was found that refluxing a 5% aqueous solution for 90 hours caused approximately 60% decomposition, calculated from the amount of liberated free acid (15).

### 5. Drug Metabolic Products

Scurr and Wyman (19) reported in 1954 that the metabolic products of trimethaphan camsylate were unknown. A search of the literature from that point up to the present did not add to the current knowledge pertaining to metabolism of the drug. Since the physiological action of the drug commences and ceases very rapidly, the effect or nature of the metabolites is uncertain.

### 6. Methods of Analysis

#### 6.1 Elemental Analysis

The results of an elemental analysis of a sample of reference standard trimethaphan camsylate are presented in Table V (16).

Table V  
Elemental Analysis of Trimethaphan Camsylate

<u>Element</u>	<u>% Theory</u>	<u>% Found</u>
C	64.40	64.39
H	6.76	6.81
N	4.69	4.76
S	10.74	10.78

### 6.2 Phase Solubility Analysis

The results of a phase solubility analysis as an indication of purity is shown in Figure 8 for a reference standard sample of trimethaphan camsylate (9). The solvent used was acetone with an equilibration time of 20 hours at 25°C. The remainder of the experimental conditions and results are shown in Figure 8.

### 6.3 Thin Layer Chromatography

A thin layer chromatographic system has been developed for the separation of hydrolysis products of trimethaphan camsylate from the parent substance (17). The type of plate used was silica gel G, while the developing solvent was methanol:10% aq.  $H_2SO_4$  (90:10). After the solvent front has ascended for 15 cm the plate is air dried and sprayed with modified Dragendorff reagent. The  $R_f$  value of trimethaphan camsylate was 0.5 while that of the major hydrolysis product was 0.7.

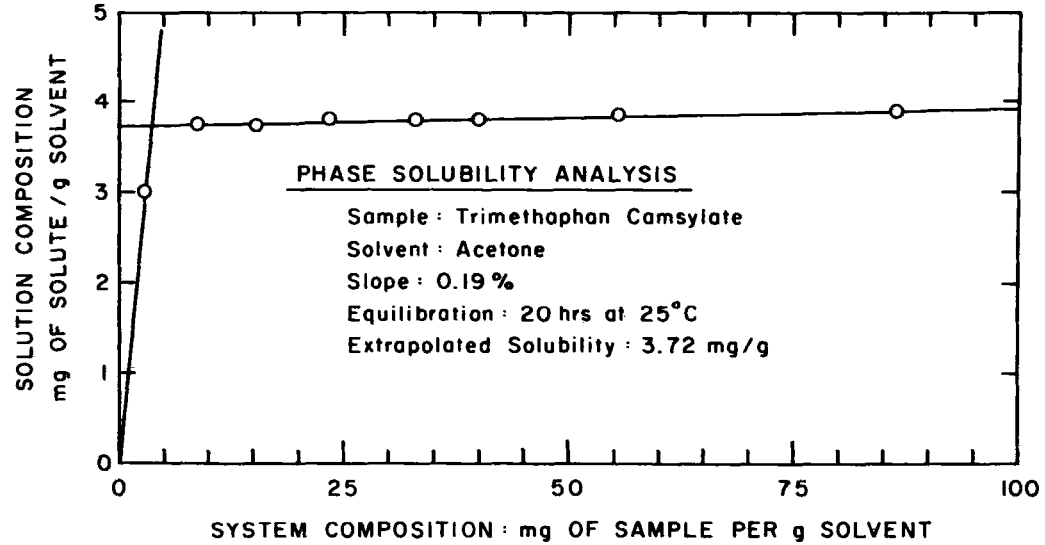
### 6.4 Direct Spectrophotometric Analysis

Trimethaphan camsylate, in injectable solution, can be assayed directly by a UV absorption procedure. This procedure involves the dilution of 5 ml of ampul solution (50 mg/cc) to one liter. The absorbance of this solution is measured at wavelengths from 254-259 nm. The maximum absorption in this range is used to calculate the amount of trimethaphan camsylate present in the ampul by comparison with a sample of reference standard material prepared and measured in a similar way (18).

### 6.5 Colorimetric Analysis

Trimethaphan camsylate can be determined colorimetrically by formation of the bromocresol green ion pair followed by extraction as described in the following procedure. A volume of solution equivalent to about 100 mg of trimethaphan camsylate is diluted to one liter. A 10-ml aliquot of this solution is buffered at a pH of 5.3 by the addition of a phosphate buffer. Then 5-ml of a bromocresol green solution in the phosphate buffer is added, after which the aqueous solution is extracted with two 25-ml portions of chloroform. The combined chloroform extracts are diluted to 100 ml and the absorbance determined at about 420 nm. The quantity of trimethaphan camsylate present is calculated by comparison with a

Figure 8



## TRIMETHAPHAN CAMSYLATE

known concentration of reference standard material similarly prepared and measured (6).

### 6.6 Non-Aqueous Titration

The non-aqueous titration described in the USP XVIII is the accepted method for the analysis of trimethaphan camsylate in the bulk form (6). The sample is dissolved in acetic anhydride and titrated with 0.1N  $\text{HClO}_4$  utilizing a potentiometric end-point. Each ml of 0.1N  $\text{HClO}_4$  is equivalent to 59.68 mg of trimethaphan camsylate.

### 7. Acknowledgment

The authors wish to acknowledge the assistance of the Research Records Office of Hoffmann-La Roche Inc. for their literature search.

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## TROPICAMIDE

*Kenneth W. Bleszel, Bruce C. Rudy, and Bernard Z. Senkowski*



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Analytical Profile - Tropicamide

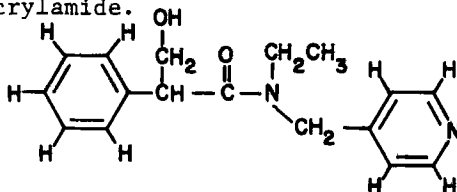
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  - 1.2 Appearance, Color, Odor
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## TROPICAMIDE

### 1. Description

#### 1.1 Name, Formula, Molecular Weight

Tropicamide is N-ethyl-2-phenyl-N-(4-pyridyl-methyl)-hydracrylamide.



$C_{17}H_{20}N_2O_2$

Molecular Weight: 284.36

#### 1.2 Appearance, Color, Odor

Tropicamide is a white crystalline odorless powder.

### 2. Physical Properties

#### 2.1 Infrared Spectrum

The infrared spectrum of a sample of reference standard tropicamide is shown in Figure 1 (1). The spectrum was recorded on a KBr pellet containing 0.5 mg of tropicamide and 300 mg of KBr, using a Perkin Elmer 621 Spectrophotometer. The following assignments have been made of the bands in Figure 1 (1).

<u>Band</u>	<u>Assignment</u>
3396 $\text{cm}^{-1}$	OH stretch
1620 $\text{cm}^{-1}$	C=O stretch
1595 and 1493 $\text{cm}^{-1}$	Aromatic Ring Vibrations
810 $\text{cm}^{-1}$	Monosubstituted Pyridine Ring
760 and 707 $\text{cm}^{-1}$	Monosubstituted Benzene Ring

#### 2.2 Nuclear Magnetic Resonance Spectrum (NMR)

The NMR spectrum of a sample of reference standard tropicamide is shown in Figure 2 (2). The sample solution contained 62.5 mg of tropicamide per 0.5 ml of  $\text{CDCl}_3$ . Due to the complex spectrum observed, extensive spin decoupling experiments were carried out in order to evaluate the coupling constants and chemical shifts. Consecutive irradiations were performed at 58.8 Hz, 65.4 Hz, 196 Hz, and 200 Hz which simplified the spectrum to the

Figure 1  
Infrared Spectrum of Tropicamide

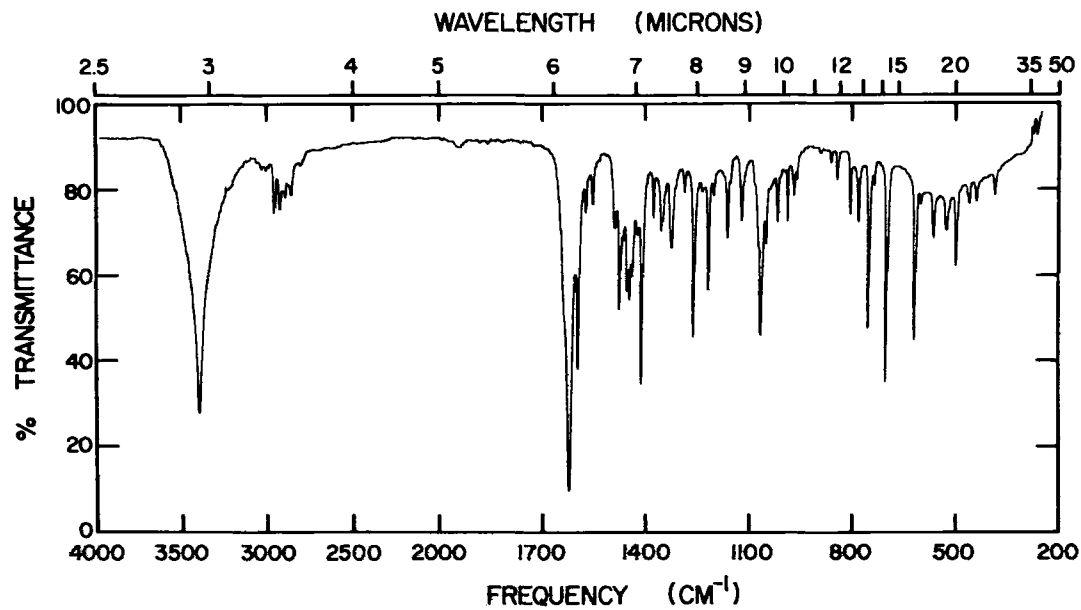
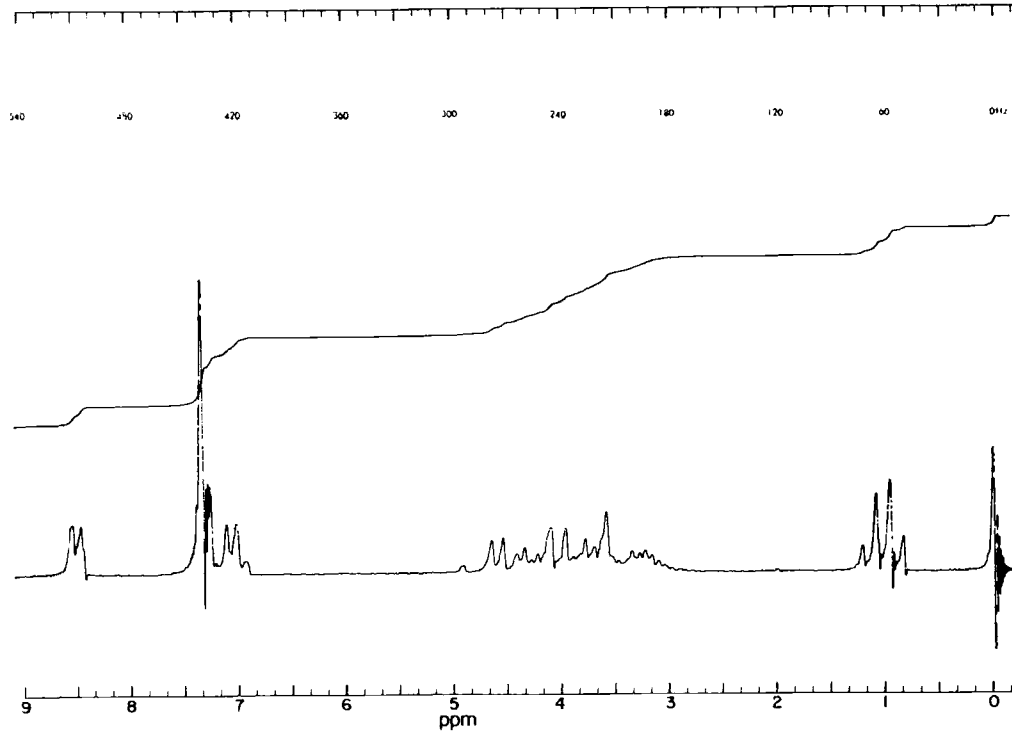


Figure 2

NMR Spectrum of Tropicamide



extent that the following assignments could be made (2).

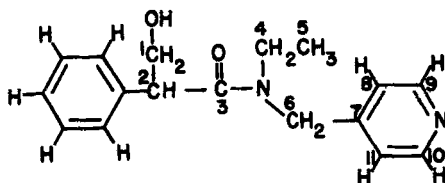


Table I

## NMR Spectral Data for Tropicamide\*

	No. of Chemical Each	Shift	Multiplicity	
C <sub>5</sub> protons	3	0.98, 1.09	(2) triplets	J[CH <sub>3</sub> -CH <sub>2</sub> -N] = 7Hz
C <sub>4</sub> protons	2	3.27	octet	J[N-CH <sub>2</sub> -CH <sub>3</sub> ] = 7Hz
OH-proton	1	3.60	singlet(broad)	
C <sub>6</sub> protons	2	3.75	multiplet	
C <sub>1</sub> and C <sub>2</sub> protons	3	~3.8-4.9	multiplet	
C <sub>8</sub> and C <sub>11</sub> protons	2	7.05	triplet	J <sub>H<sub>8</sub>-H<sub>9</sub></sub> = 5Hz
aromatic protons on tropic	5	7.35		
acid moiety				
C <sub>9</sub> and C <sub>10</sub> protons	2	8.50	doublet	J <sub>H<sub>9</sub>-H<sub>8</sub></sub> = 5Hz

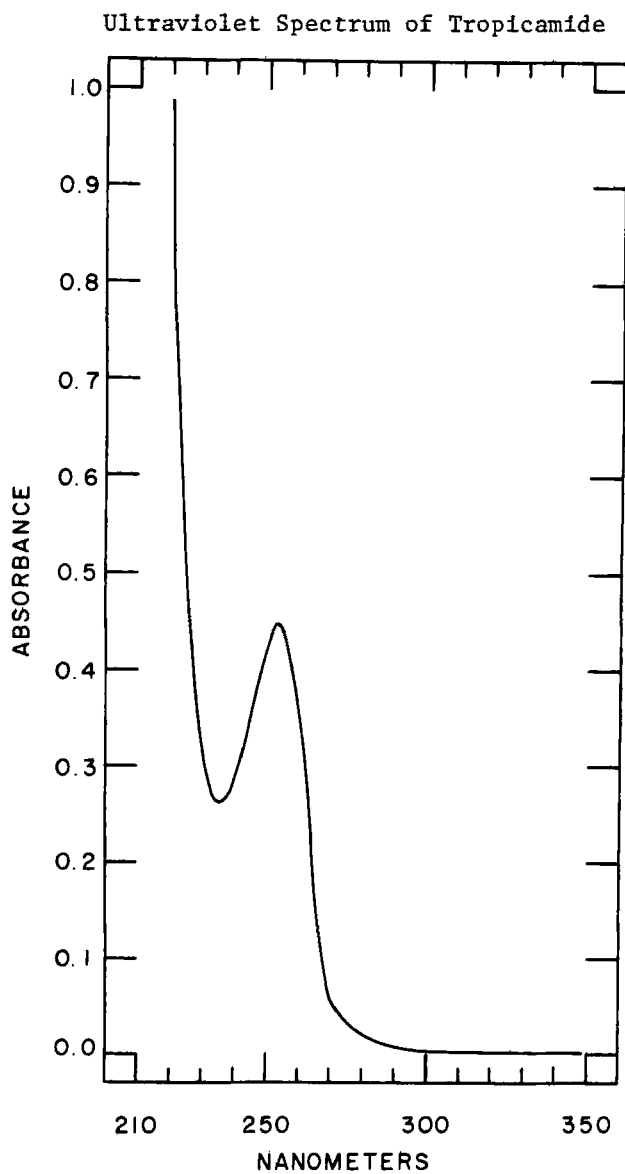
\* Arbitrary numbers were assigned to the atoms in the structure for ease in presentation of data.

### 2.3 Ultraviolet Spectrum

The ultraviolet spectrum of tropicamide in the region of 200-400 nm is shown in Figure 3 (3). The spectrum shows a maximum at 254 nm ( $\epsilon = 5.1 \times 10^3$ ) and a minimum at 235-237 nm. The solution concentration was 0.025 mg/ml in 0.1N HCl.

TROPICAMIDE

Figure 3



#### 2.4 Fluorescence Spectrum

An excitation and emission scan were carried out with a methanol solution of reference standard tropicamide. There was, however, no fluorescence observed (4).

#### 2.5 Mass Spectrum

The low-resolution mass spectrum of tropicamide is shown in Figure 4 (5). The spectrum was obtained using a CEC 21-110 spectrometer with an ionizing voltage of 70 eV, which was interfaced with a Varian data system 100 MS. The data system accepted the output of the spectrometer, calculated the masses, compared their intensities to the base peak and plotted this information as a series of lines whose heights were proportional to the intensities.

The molecular ion was measured at  $m/e$  284. The base peak at  $m/e$  254 arises due to the loss of  $\text{CH}_2 = 0$  from the molecular ion by a McLafferty rearrangement. The ion at  $m/e$  225 probably arises via a skeletal rearrangement of  $m/e$  254 leading to the loss of  $\text{HCO}$ . Cleavage between the benzyl carbon and the carbonyl group gives rise to the ion at  $m/e$  163. The ion at  $m/e$  92 is the nitrogen-containing tropylium cation,  $\text{C}_6\text{H}_6\text{N}^+$  (5).

#### 2.6 Optical Rotation

Tropicamide does not exhibit optical activity.

#### 2.7 Melting Range

The melting range reported in the United States Pharmacopeia XVIII for tropicamide is  $96\text{--}100^\circ\text{C}$  when a Class I procedure is used (6).

#### 2.8 Differential Scanning Calorimetry (DSC)

The DSC scan of a sample of reference standard tropicamide is shown in Figure 5 (7). The temperature was raised at a rate of  $10^\circ\text{C}/\text{min}$ . in an atmosphere of flowing nitrogen. A single endotherm was observed, the extrapolated onset of which was  $95.5 \pm 0.2^\circ\text{C}$ . The peak of the melting endotherm was observed at  $98.6 \pm 0.2^\circ\text{C}$ . All temperatures are corrected. The value of  $\Delta H_f$  for the melting endotherm was calculated to be  $8.8 \text{ kcal/mole}$ .

#### 2.9 Thermogravimetric Analysis (TGA)

The results of a TGA scan of tropicamide indicated that no weight loss occurred from ambient temperature

Figure 4  
Mass Spectrum of Tropicamide

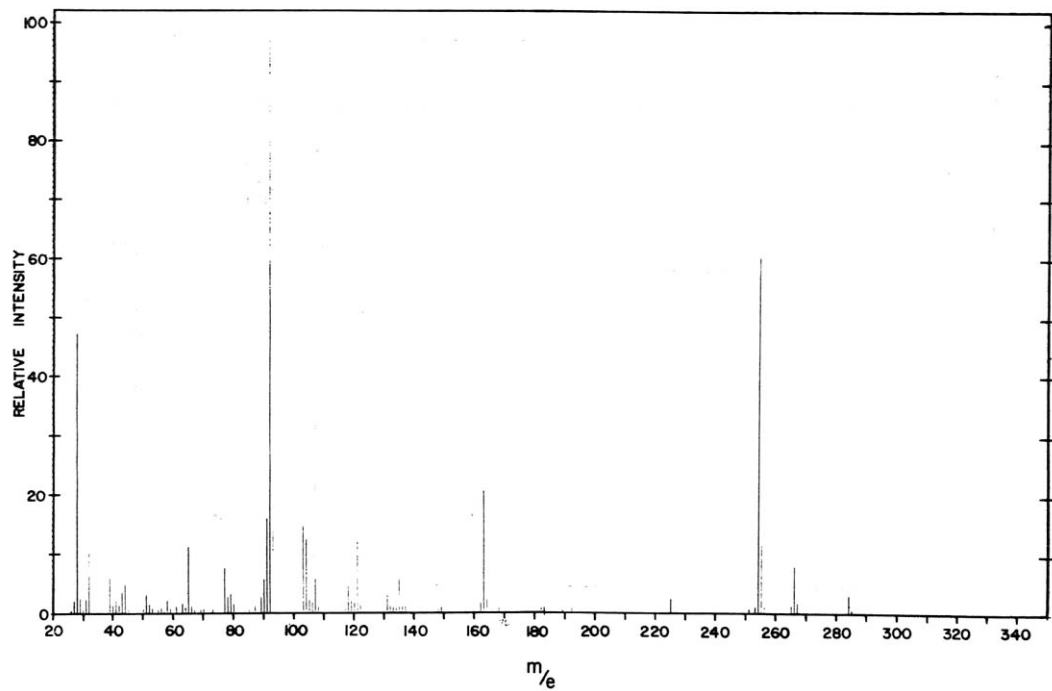
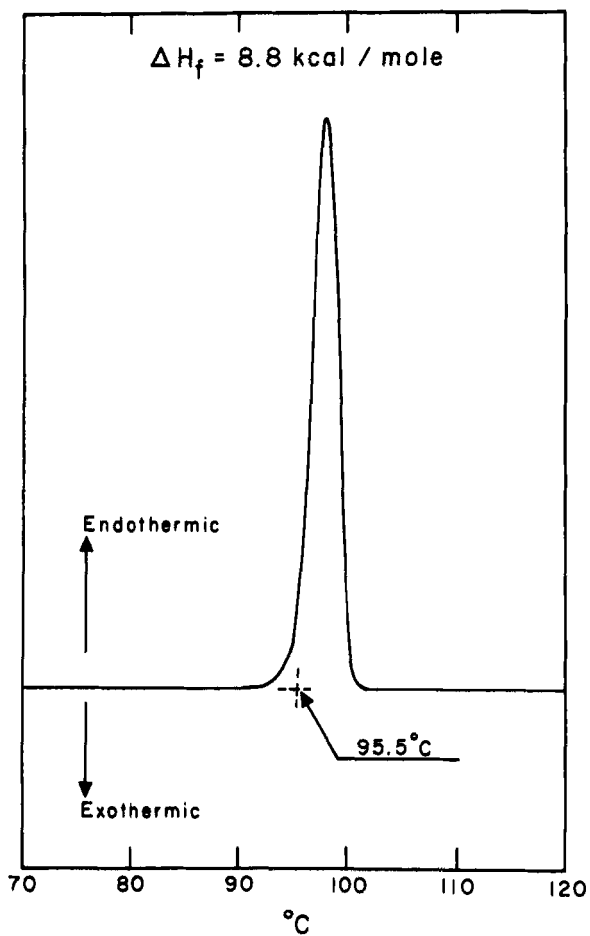




Figure 5

DSC Curve for Tropicamide



## TROPICAMIDE

to 150°C. A single weight loss was observed beginning at 150°C and continuing to 334°C at which point 100% of the sample weight had been lost (7).

### 2.10 Solubility

The solubility data shown in Table II was obtained for a sample of reference standard tropicamide at a temperature of 25°C (8).

Table II

#### Solubilities of Tropicamide

<u>Solvent</u>	<u>Solubility (mg/ml)</u>
3A alcohol	235.0
benzene	25.9
chloroform	>500.
95% ethanol	321.5
diethyl ether	3.9
2-propanol	112.4
methanol	>500.
petroleum ether (30-60°)	0.2
water	5.7

### 2.11 Crystal Properties

Table III gives interplanar spacings from x-ray powder diffraction data for tropicamide (9). The operating parameters of the instrument are given below.

#### Instrumental Conditions:

##### General Electric Model XRD-6 Spectrogoniometer

Generator:	50 KV, 12-1/2 MA
Tube target:	Copper
Radiation:	Cu $K_{\alpha}$ = 1.542 Å
Optics:	0.1° Detector slit 3° Beam slit 0.0007" Ni filter 4° take off angle
Goniometer:	Scan at 0.2° 2θ per minute

Detector: Amplifier gain - 16 course,  
 8.7 fine  
 Sealed proportional counter  
 tube and DC voltage at  
 plateau  
 Pulse height selection  $E_L$  -  
 5 volts  
 $E_U$  - out  
 Rate meter T.C. 4  
 2000 C/S full scale  
 Recorder: Chart speed 1 inch per 5  
 minutes  
 Samples: Prepared by grinding at room  
 temperature

Table III

## Interplanar Spacings from Powder Diffraction Data

$2\theta$	$d^*$	$I/I_0^{**}$	$2\theta$	$d^*$	$I/I_0^{**}$
10.54	8.39	9	29.74	3.00	11
13.76	6.44	75	30.06	2.97	12
14.60	6.07	63	31.42	2.85	4
18.14	4.89	27	32.00	2.80	8
18.94	4.69	47	33.06	2.71	5
19.84	4.47	12	34.46	2.60	9
20.40	4.35	67	35.18	2.55	8
20.88	4.25	72	35.52	2.53	15
21.88	4.06	100	37.04	2.43	4
22.60	3.93	28	37.54	2.40	4
23.22	3.83	13	39.00	2.31	7
24.30	3.66	19	39.82	2.26	4
25.38	3.51	12	40.22	2.24	8
26.10	3.41	4	40.62	2.22	4
26.46	3.37	4	41.66	2.17	2
27.54	3.24	30	42.24	2.14	4
28.00	3.19	5	42.70	2.12	3
28.94	3.09	41	45.54	1.99	6

$$* d = (\text{interplanar spacing}) \frac{n\lambda}{2 \sin \theta}$$

$** I/I_0$  = relative intensity (based on highest  
 intensity of 100)

## TROPICAMIDE

### 2.12 Dissociation Constant

The pKa of tropicamide was determined by spectrophotometric analysis and by a potentiometric titration. The value observed was 5.2 by spectrophotometry and 5.3 by potentiometry (10).

### 3. Synthesis

Tropicamide may be prepared by the condensation of ethyl-( $\gamma$ -picolyl)-amine with tropic acid chloride, in the presence of base, carried out in anhydrous chloroform (11).

### 4. Stability Degradation

A study has been carried out in which the stability of tropicamide in ophthalmic solution was determined (12). A 3% solution of tropicamide in ophthalmic solution was maintained at temperatures ranging from 0°C to 45°C for periods of time up to 12 weeks. In order to gain information about possible breakdown products of tropicamide, pH measurements, turbidity data and a direct spectrophotometric assay was performed at the start and after 3, 6 and 12 weeks. No evidence of decomposition was found after periods of up to 12 weeks at each of the above temperatures (12).

### 5. Drug Metabolic Products

Tropicamide is used exclusively for ophthalmic solutions in this country, and is applied topically. For this reason no metabolic studies have been pursued.

### 6. Methods of Analysis

#### 6.1 Elemental Analysis

The results of an elemental analysis of a sample of reference standard tropicamide are presented in Table IV (13).

<u>Element</u>	<u>% Theory</u>	<u>% Found</u>
C	71.81	71.87
H	7.09	7.13
N	9.85	9.93

#### 6.2 Phase Solubility Analysis

Phase solubility analyses have been carried out for tropicamide to estimate the purity of the sample. An

example is shown in Figure 6 (8) where the solvent used was toluene and the equilibration time was 20 hours at 25°C.

#### 6.3 Thin Layer Chromatographic Analysis

A TLC system has been developed which has proved to be useful for analysis of tropicamide. The adsorbant for the system is silica gel and the developing solvent is chloroform:methanol:concentrated ammonium hydroxide (90:10:2). The solvent front is allowed to travel for about 15 cm in a pre-saturated tank. The plate is air dried and then sprayed with iodine-modified Dragendorff reagent. The approximate  $R_f$  of tropicamide in this system is 0.65 (14).

#### 6.4 Direct Spectrophotometric Analysis

Tropicamide may be assayed spectrophotometrically in opthalmic solution after an extraction into chloroform and a back extraction into dilute sulfuric acid. The absorbance of this solution is measured at the wavelength of maximum absorbance at about 253 nm. The amount of tropicamide in the opthalmic solution is calculated by comparison with a reference standard sample of tropicamide measured in a similar way (6).

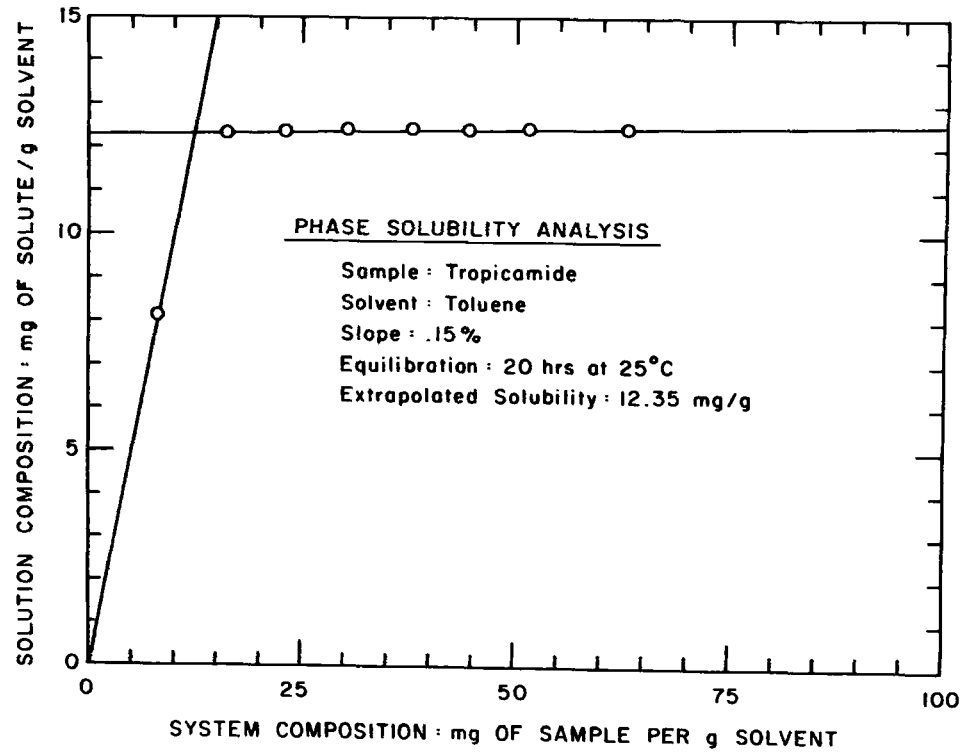
#### 6.5 Non-Aqueous Titration

The non-aqueous titration described in the USP XVIII is the preferred method for the analysis of tropicamide in the bulk form (6). The sample is titrated in glacial acetic acid with 0.1N  $\text{HClO}_4$  in acetic acid, using crystal violet as the indicator. One ml of 0.1N  $\text{HClO}_4$  is equivalent to 28.44 mg of tropicamide.

#### 7. Acknowledgments

The authors wish to acknowledge the Scientific Literature Department and the Research Records Office of Hoffmann-La Roche Inc. for their assistance in the literature search for this analytical profile.

Figure 6



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